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Ochiai

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(45) **Date of Patent: Sep. 27, 2016**

(54) **PHOSPHATIDIC ACID PHOSPHATASE GENE AND USE THEREOF**

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(30) **Foreign Application Priority Data**

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(51) **Int. Cl.**

C07H 21/04	(2006.01)
C12P 21/02	(2006.01)
C12P 7/64	(2006.01)
C12N 9/16	(2006.01)
C12N 15/63	(2006.01)
C12N 5/10	(2006.01)
C12N 1/15	(2006.01)
C12N 1/19	(2006.01)
C12N 1/21	(2006.01)
C12N 15/82	(2006.01)

(52) **U.S. Cl.**

CPC ***C12N 9/16*** (2013.01); ***C12N 15/8247*** (2013.01); ***C12P 7/6463*** (2013.01); ***C12Y 301/03004*** (2013.01); *Y02P 20/52* (2015.11)

(58) **Field of Classification Search**

None

See application file for complete search history.

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Primary Examiner — Xiaozhen Xie

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(57) **ABSTRACT**

The present invention provides phosphatidic acid phosphatase cDNAs and recombinant vectors comprising nucleic acids encoding proteins having phosphatidic acid phosphatase activity wherein 100 amino acids at the N-terminal region and DXDX(T/V) catalytic site motif are conserved in the protein.

5 Claims, 19 Drawing Sheets

Figure 1-1

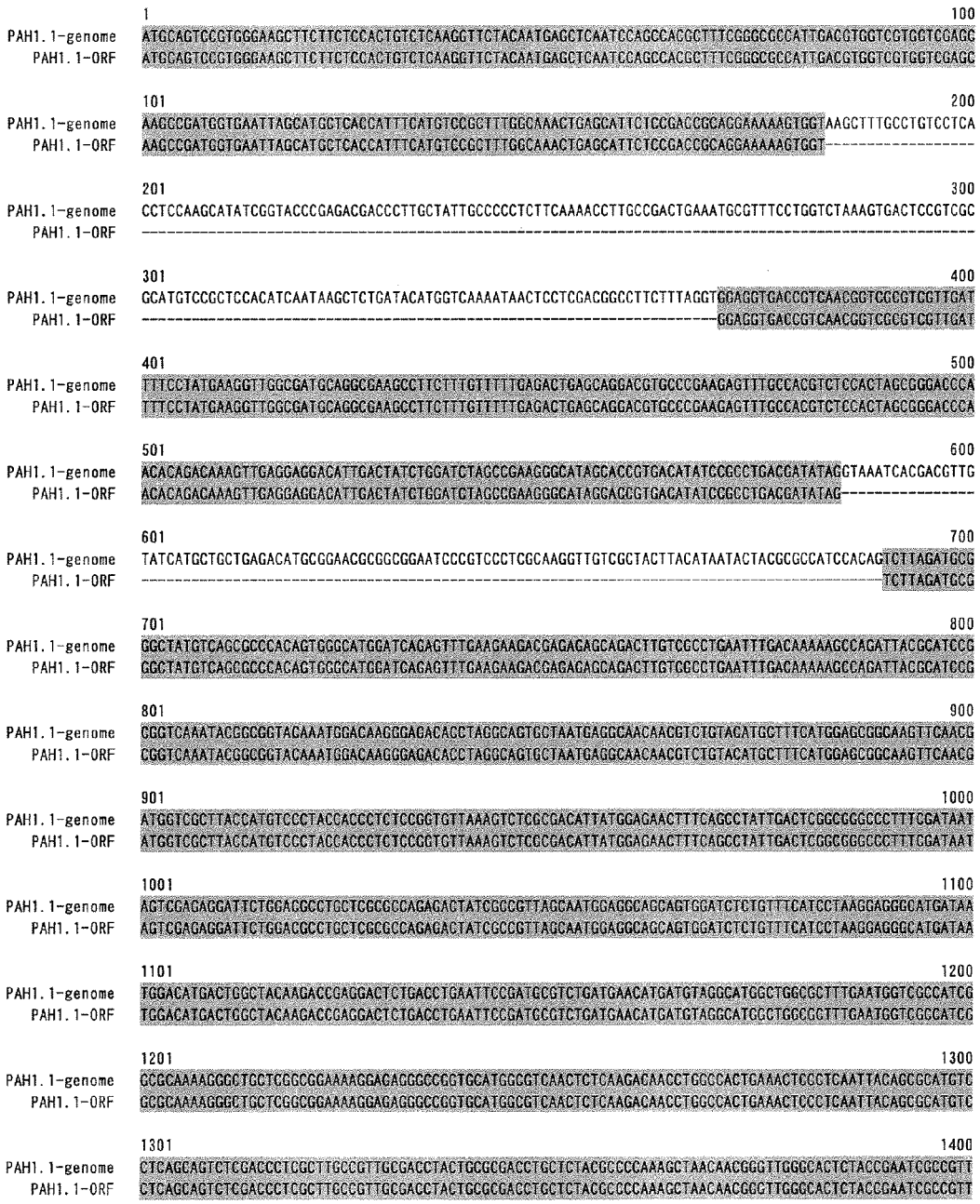
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Figure 1-2

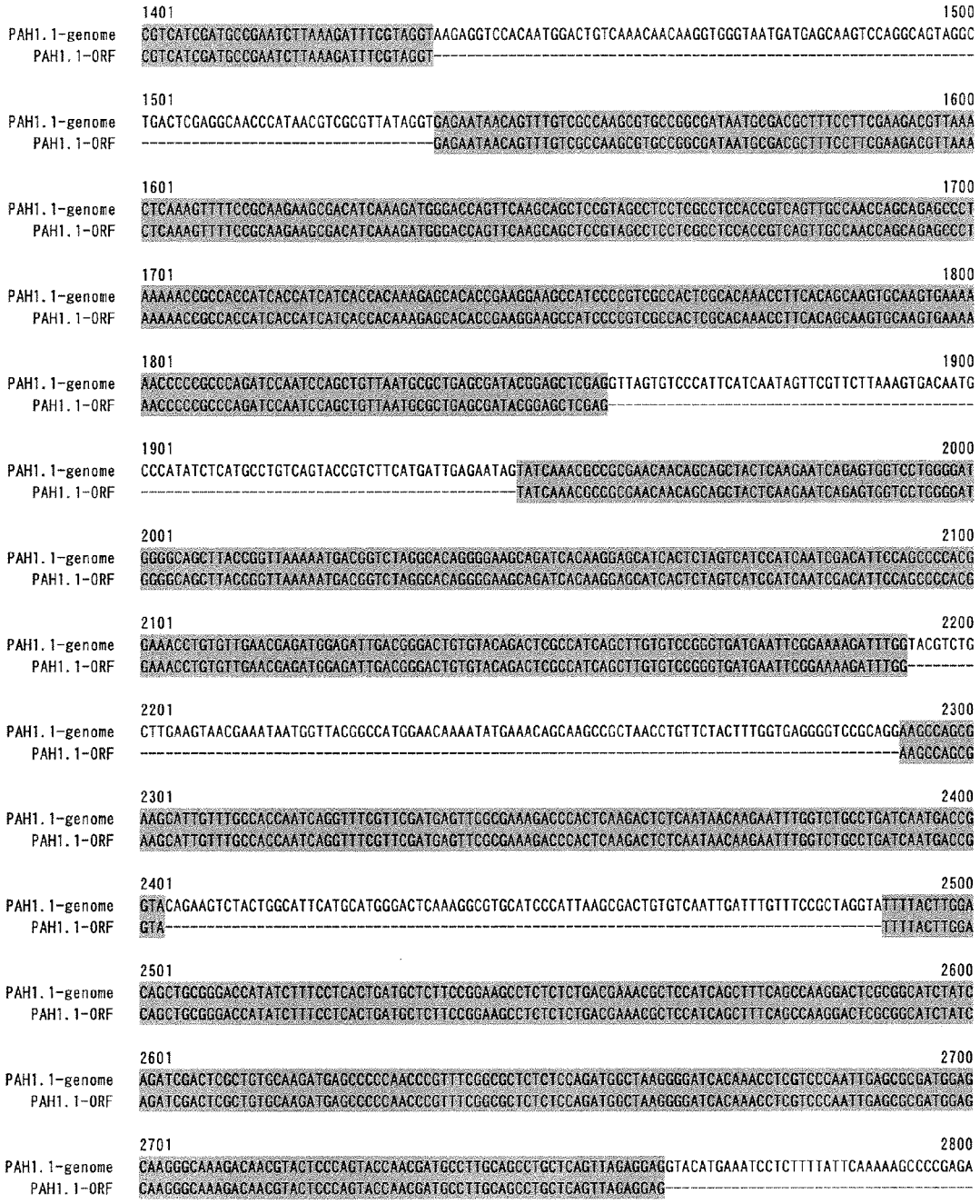
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Figure 1-3

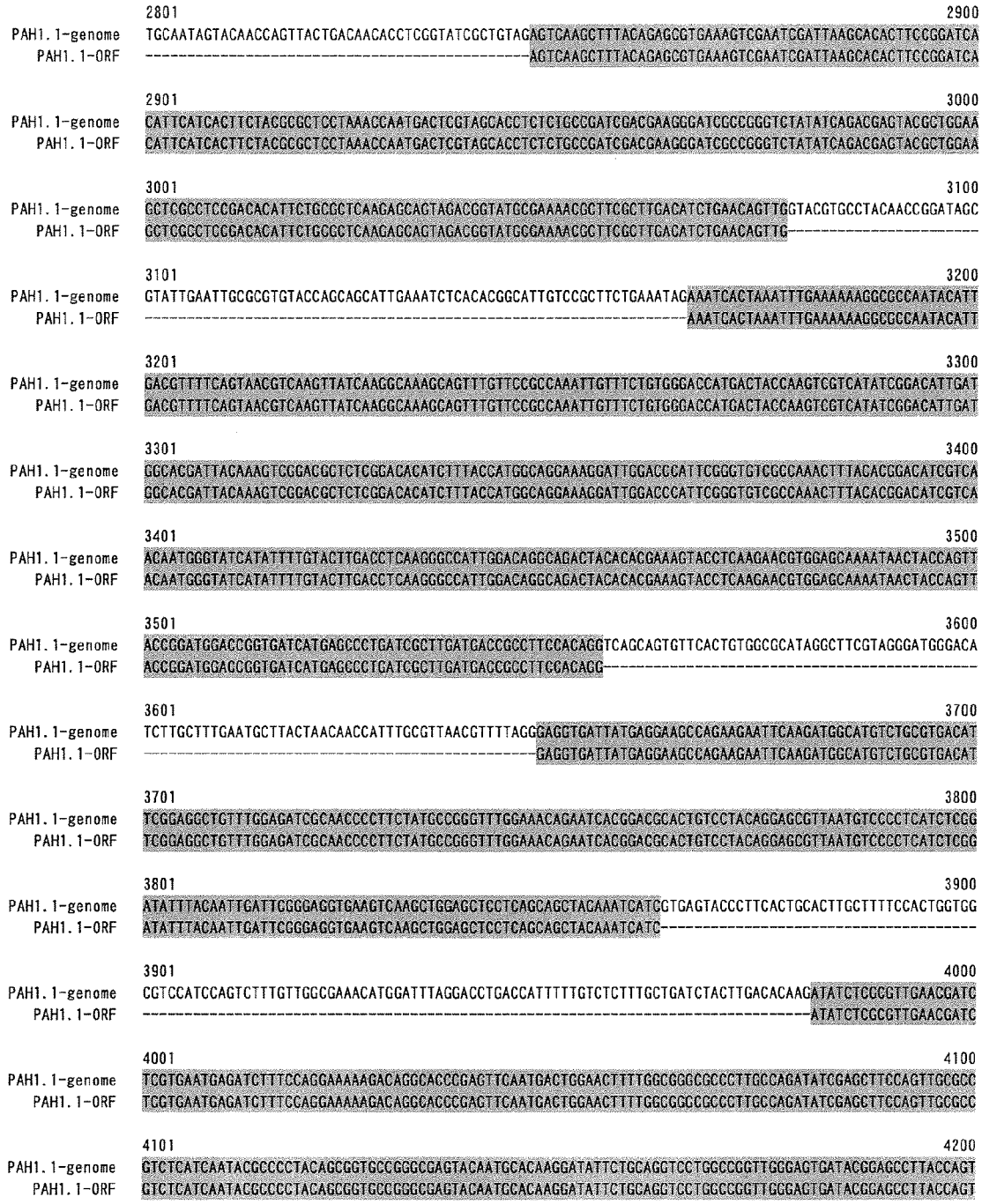
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Figure 1-4

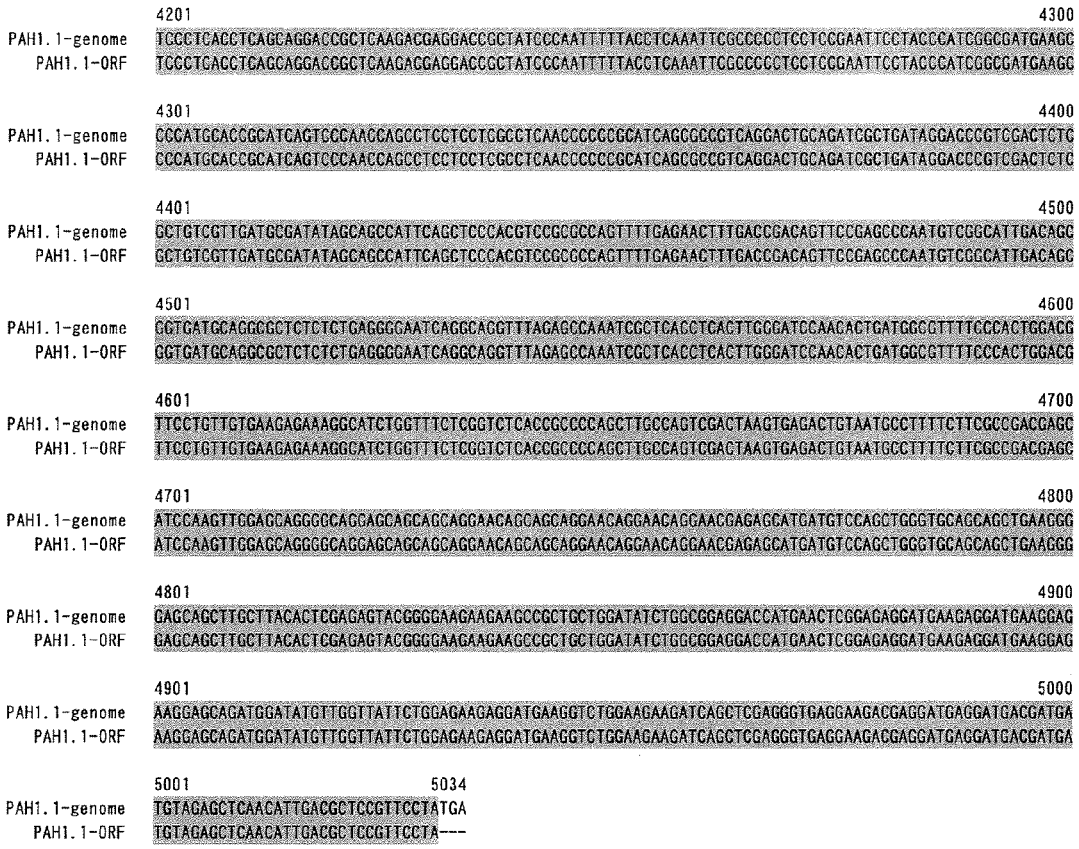
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Figure 2-1

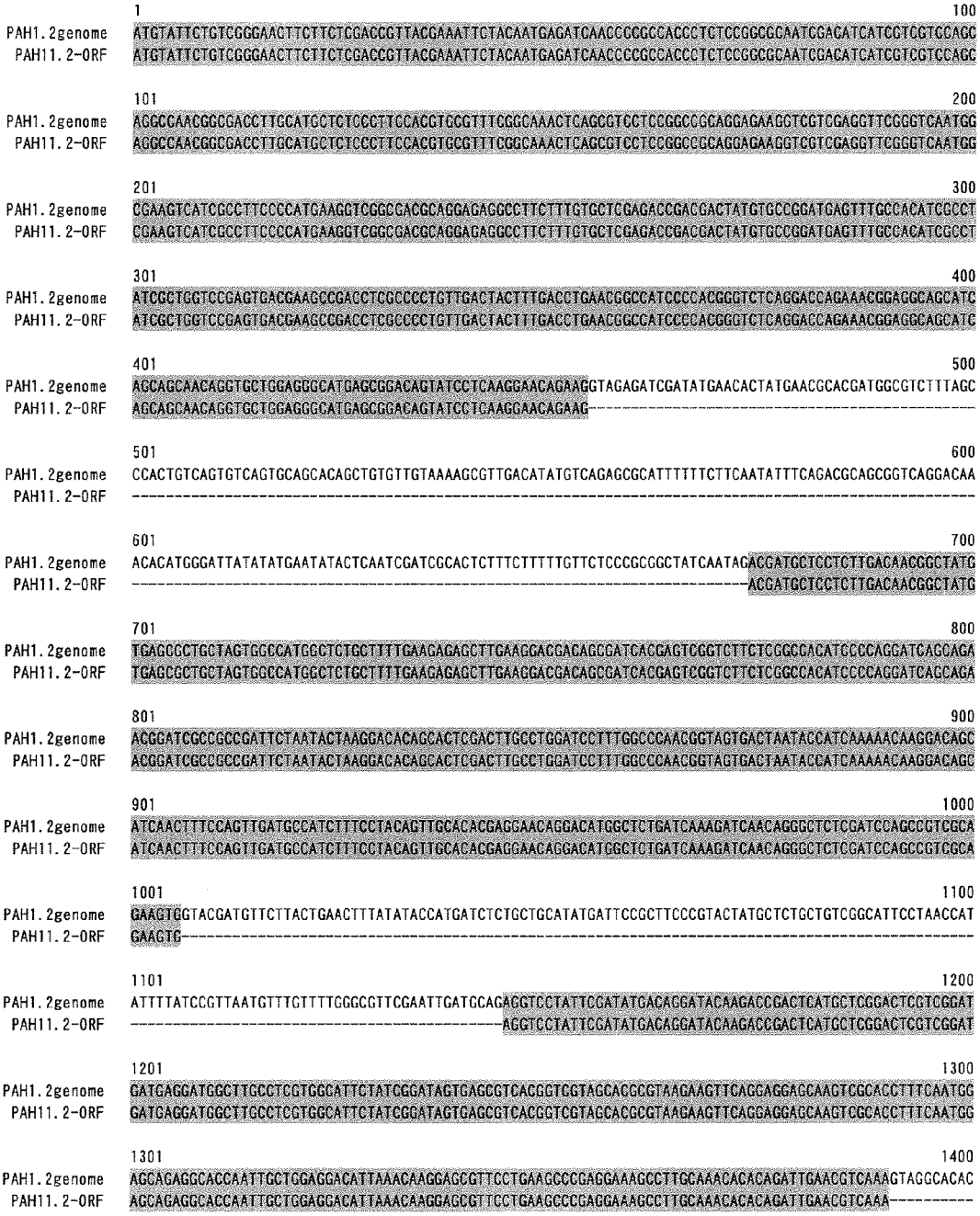
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Figure 2-2

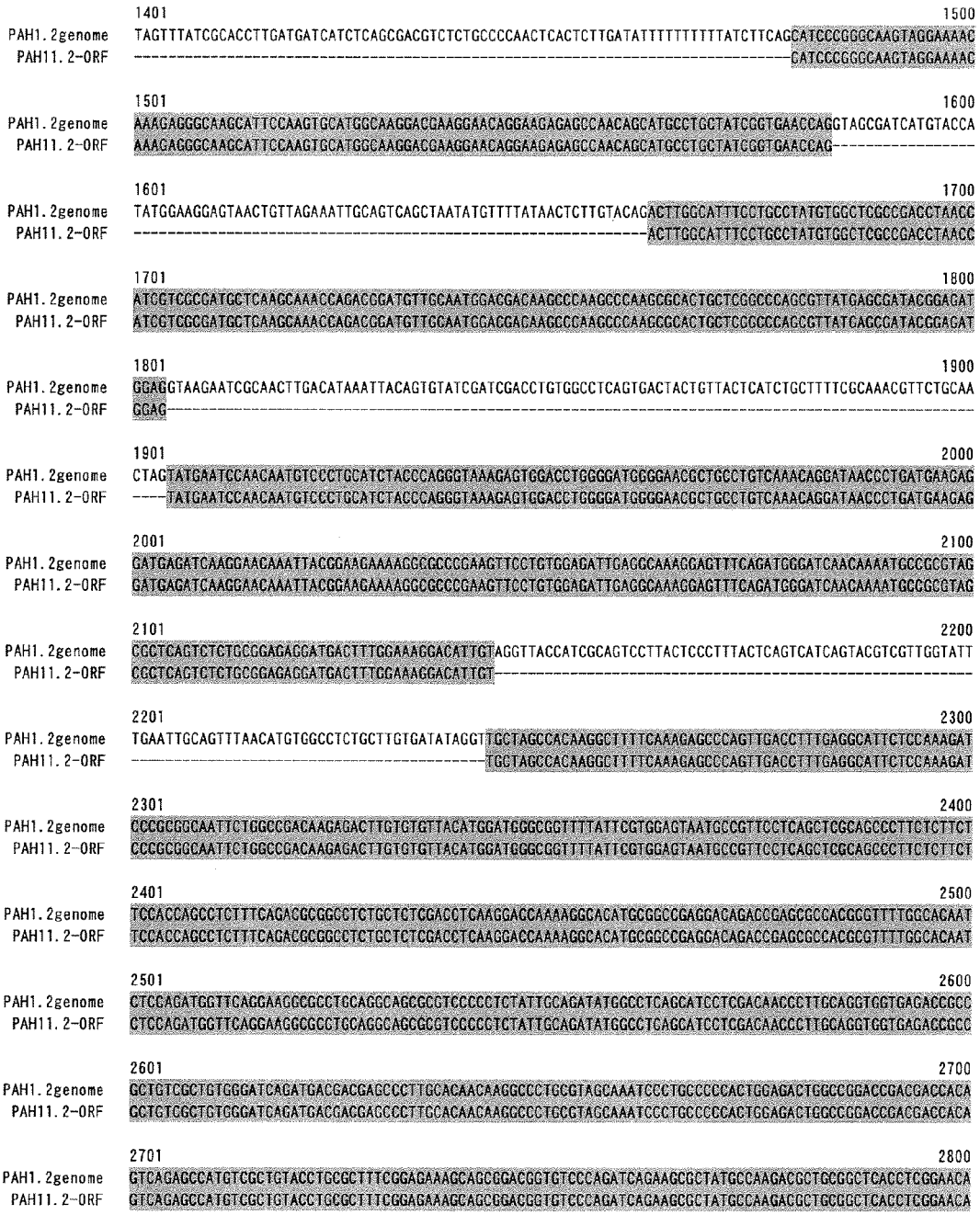
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Figure 2-3

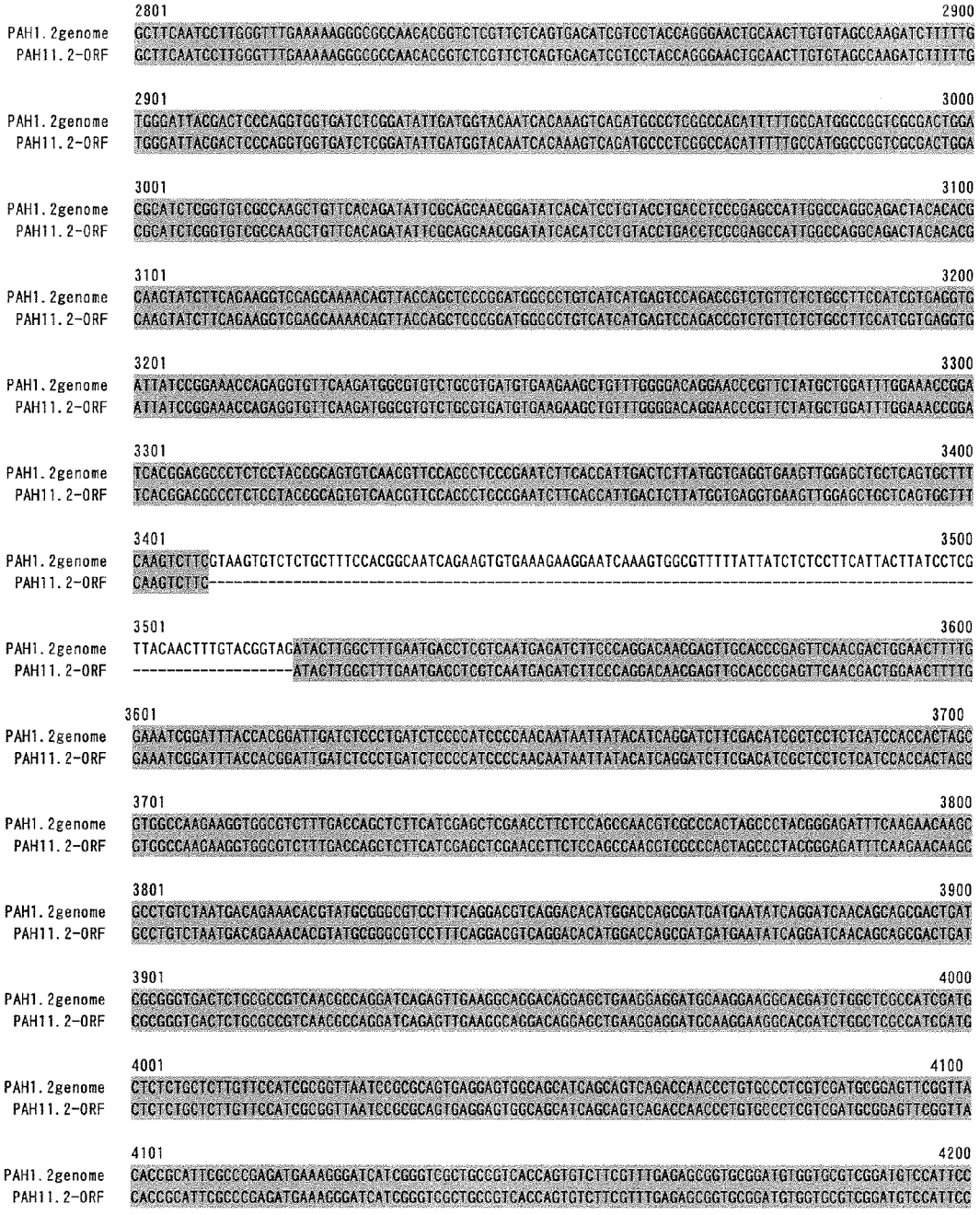
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Figure 2-4


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Figure 3-1

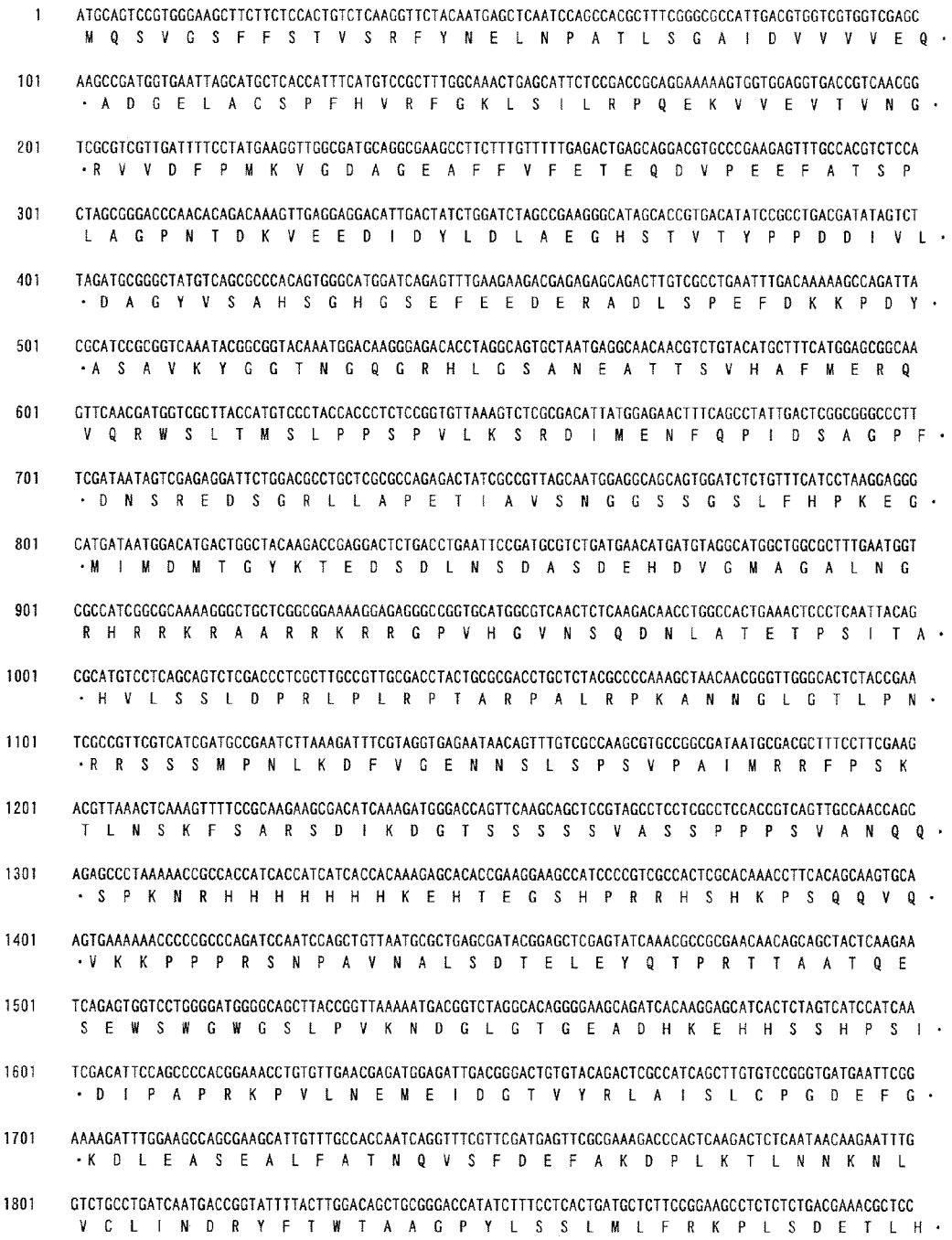
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Figure 3-2

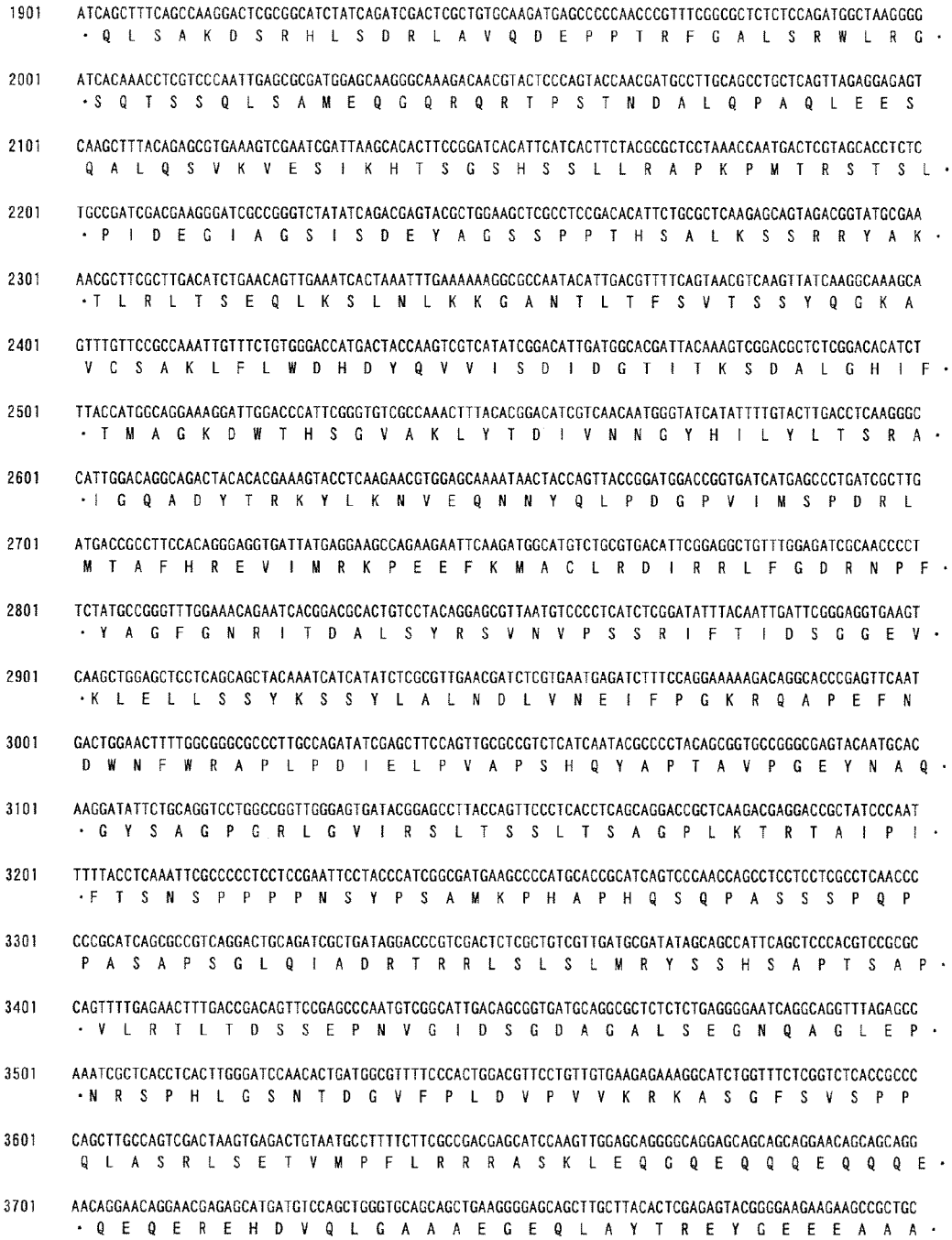
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Figure 3-3

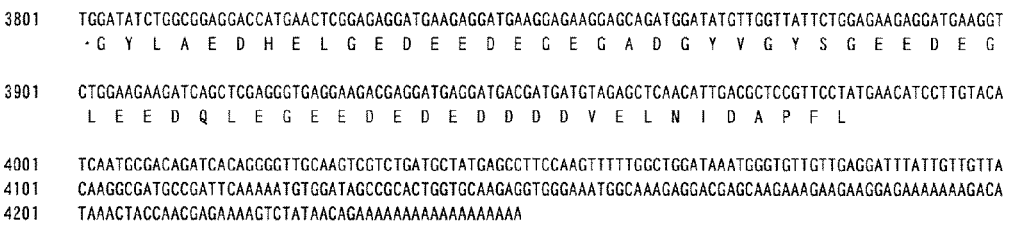
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Figure4-1

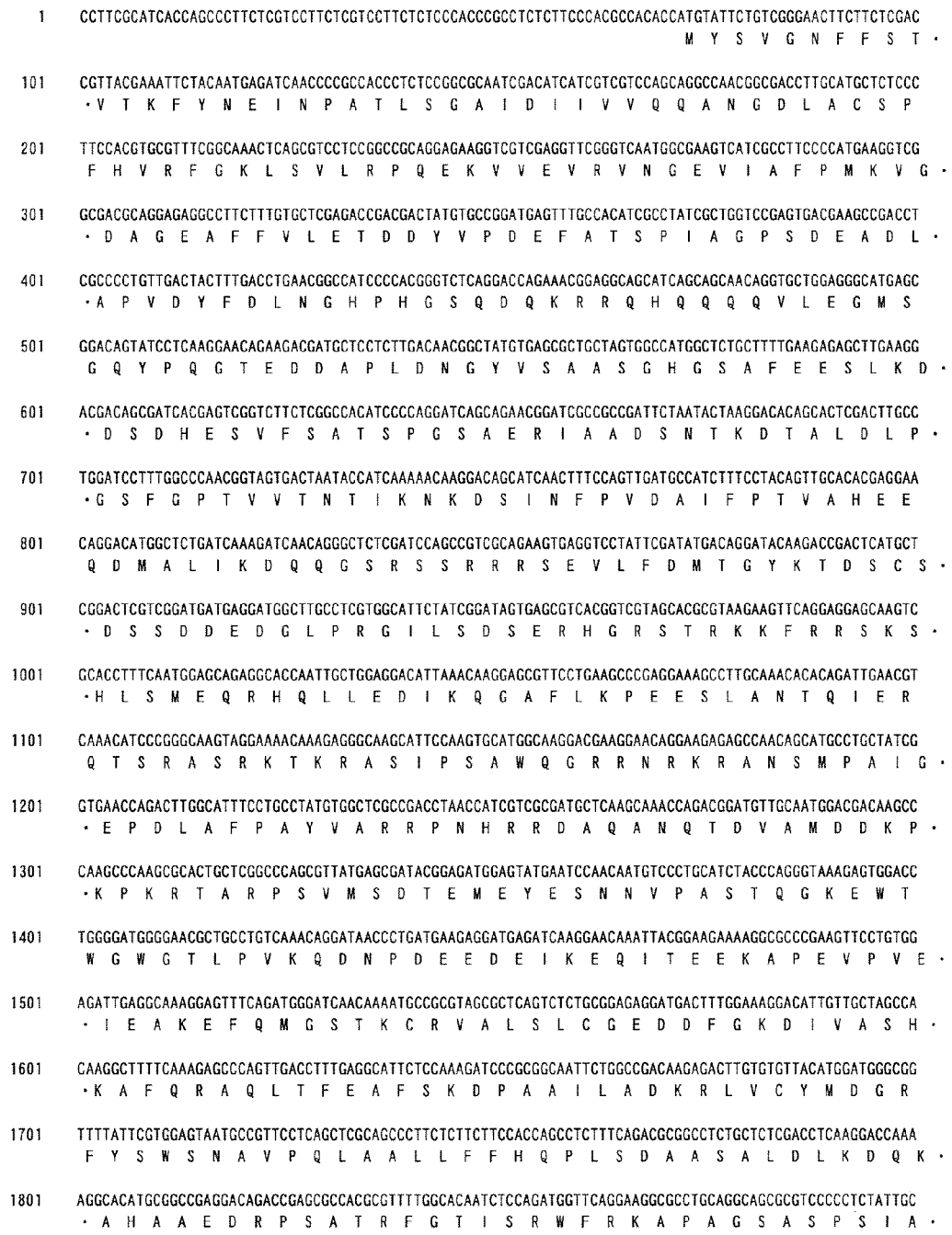
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Figure 4-2

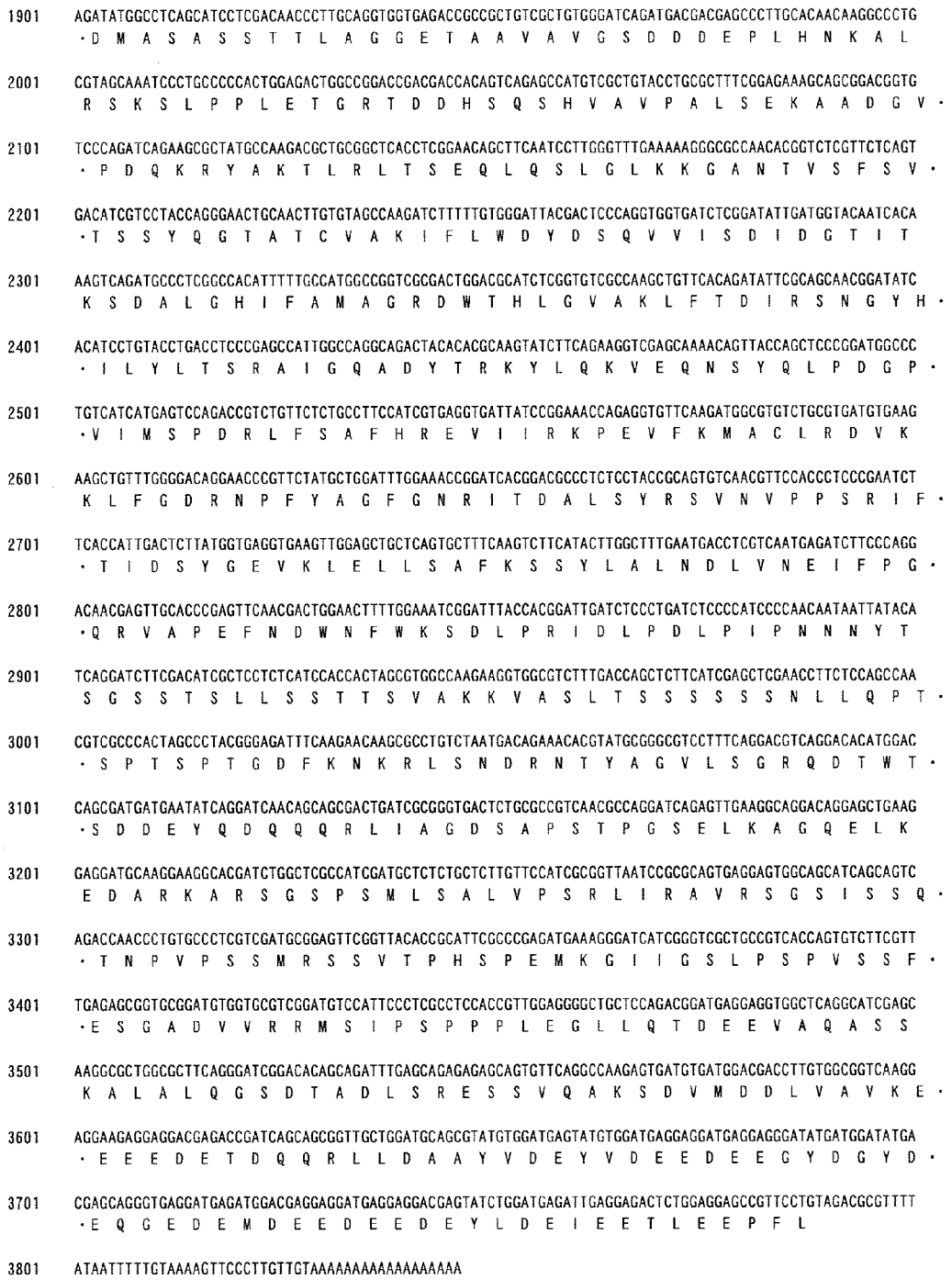
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Figure 5-1

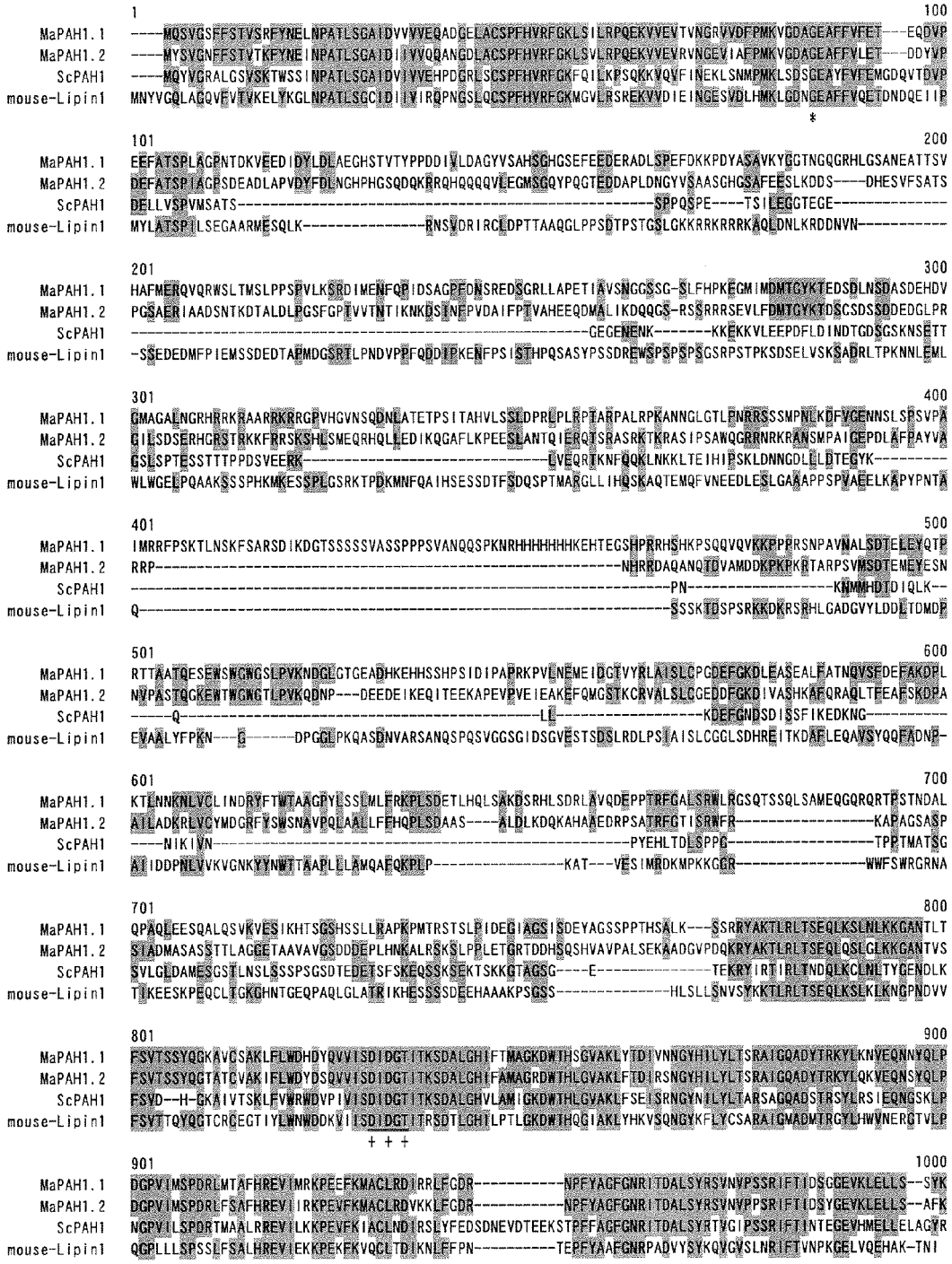

Figure 5-2

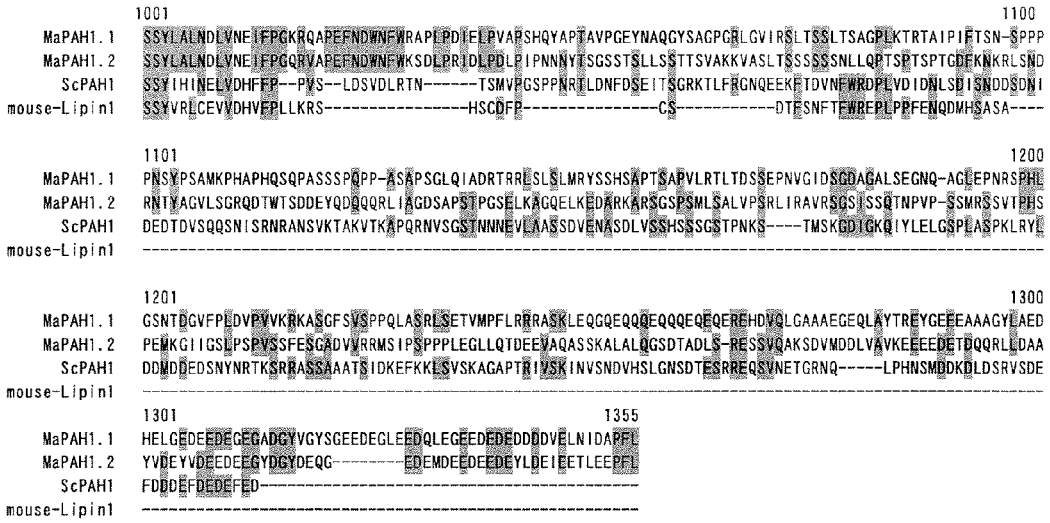
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Figure 6-1

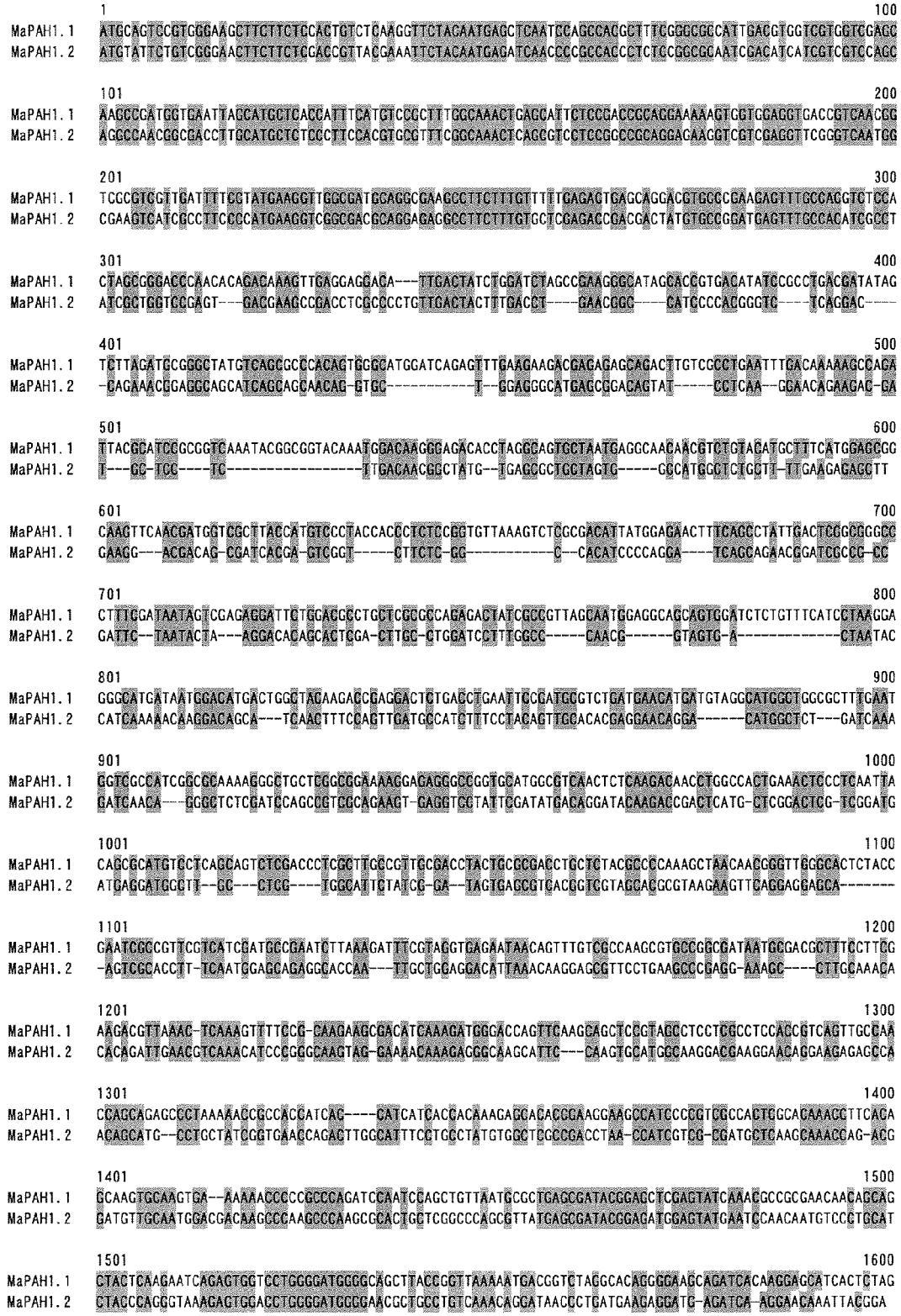
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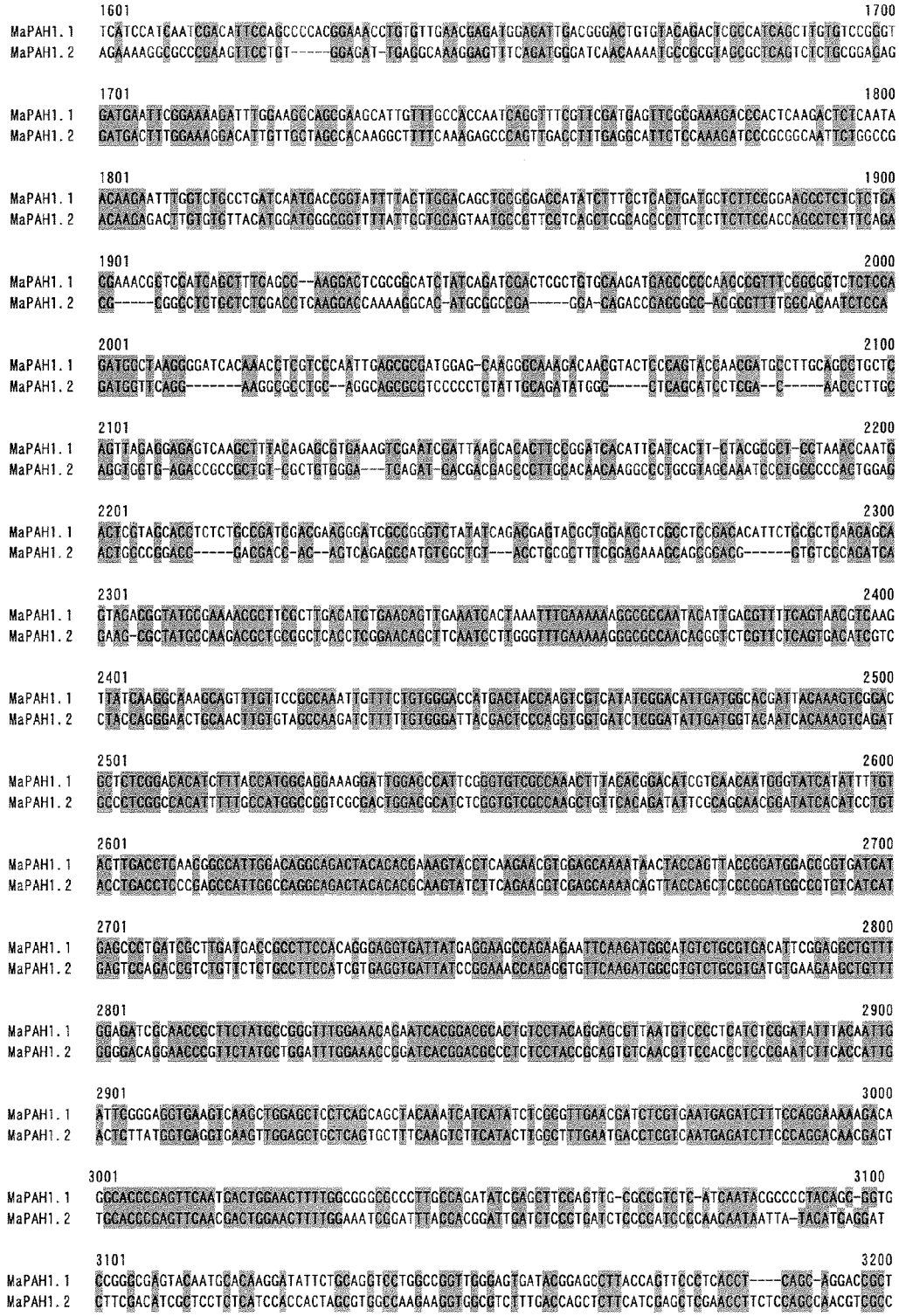
Figure 6-2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3101 3200
MaPAH1.1 CCGGCCGAGTACAATGCACAAGGATATTCTGCAGGTCCTGGCCGGTTGGGAGTGATACGGAGCCTTACCAGTTCCCTCACCT-----CAGC-AGGACCGCT
MaPAH1.2 GTTCGACATCGCTCCTGTCATCCACCACTAGCGTGGCCAAGAAGGTGCCGTCTTTGACCAGCTCTTCATCGAGCTCGAACCTTCTCCAGCCAACGTCGCC

Figure 6-3

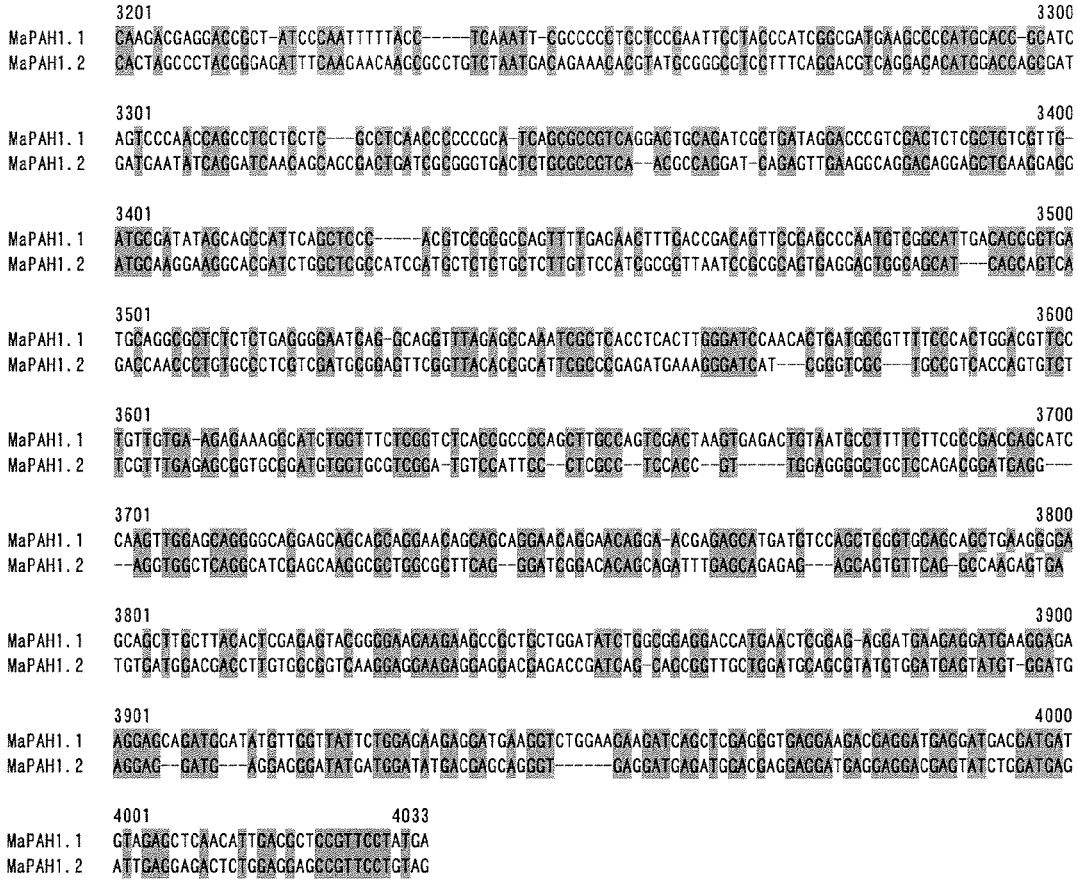
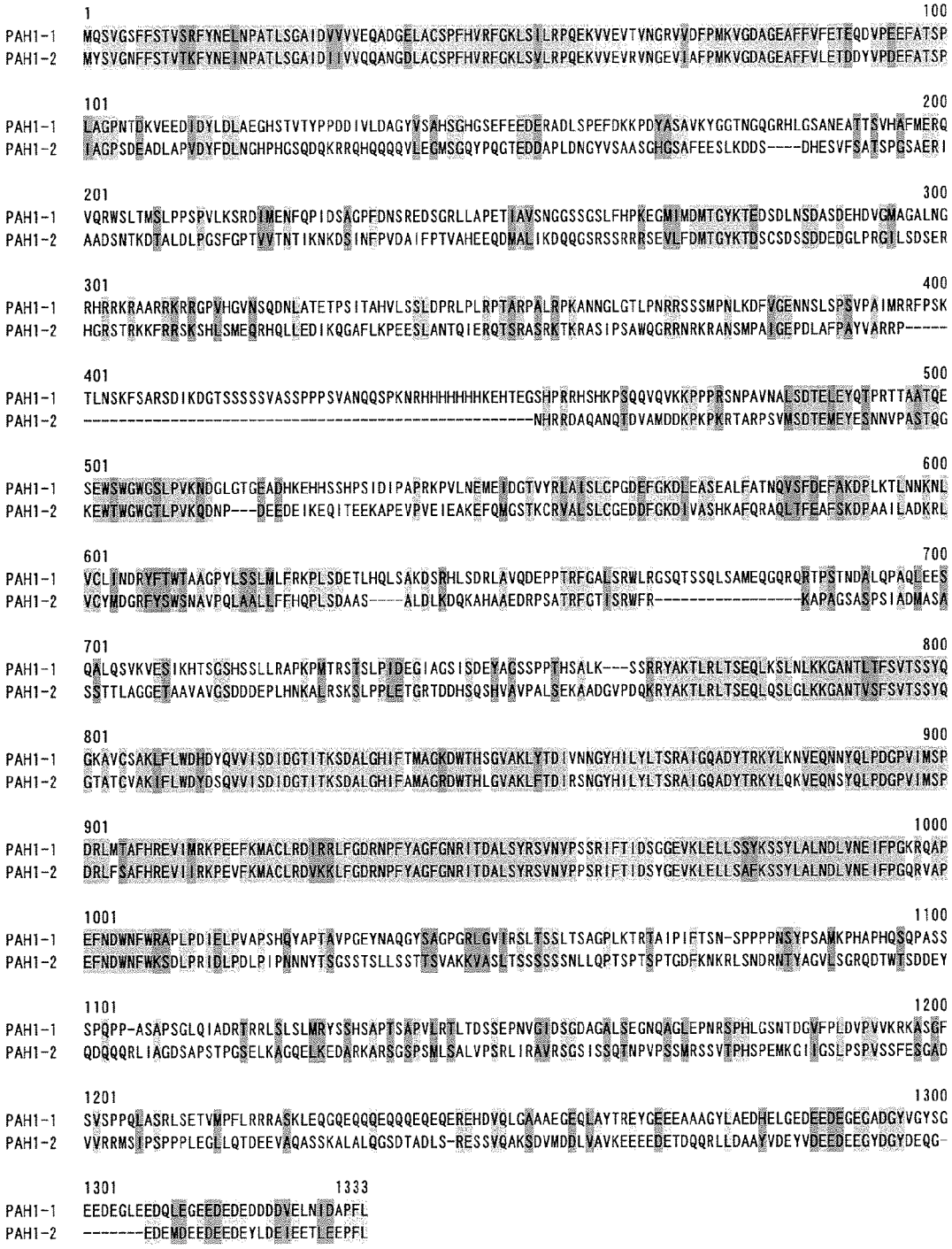

3201
3300
MaPAH1.1 CAACACGAGGACCGCT-ATCCCAATTTTTACC-----TCAAATT-CGCCCCCTCCTCCGAATTCCTACCCATCGGCGATGAAGCCCCATGCACG-GCATC
MaPAH1.2 CACTAGCCCTACGGGAGATTTCAAGAACAAGCGCCTGTCTAATGACAGAAACACGTATGCGGGCCTCCTTTCAGGACGTCAGGACACATGCACCAGCGAT
3301
3400
MaPAH1.1 AGTCCCAACCAGCCTCCTCCTC----GCCTCAACCCCCCGCA-TCAGCGCCGTCAGGACTGCAGATCGCTGATAGGACCCGTCGACTCTCGCTGTCGTTG-
MaPAH1.2 GATCAATATCAGGATCAACAGCAGCGACTGATCGCGGGTGACTCTGCGCCGTCA--ACGCCAGGAT-CAGAGTTGAAGGCAGGACAGGAGCTGAAGGAGC
3401
3500
MaPAH1.1 ATGCGATATAGCAGCCATTCAGCTCCC------ACGTCCGCGCCAGTTTTGAGAAGTTTGACCGACAGTTCCGAGCCCAATGTCGGCATTGACACGCGTCA
MaPAH1.2 ATGCAAGGAAGGCAGGATCTGGCTCGCCATCCATGCTCTGTGCTCTTGTTCCATCGCCGTTAATCCGCGCAGTGAGGACTGGCAGCAT----CAGCAGTCA
3501
3600
MaPAH1.1 TGCAGGCGCTCTCTCTGAGGGGAATCAG-GCAGGTTTAGAGCCAAATCGCTCACCTCACTTGGGATCCAACACTGATGGCGTTTTCCCACTGCACGTTCC
MaPAH1.2 GACCAACCCTGTGCCCTCGTCGATGCGGAGTTCGCTTACACCGCATTCGCCCGAGATGAAAGGGATCAT----CGGGTCCC----TGCCGTCACCAGTGTCT
3601
3700
MaPAH1.1 TGTTGTGA-AGAGAAAGGCATCTGGTTTCTCGGTCTCACCGCCCCAGCTTGCCAGTCGACTAAGTGAGACTGTAATGCCTTTTCTTCGCCGACGAGCATC
MaPAH1.2 TCGTTTGAGAGCGGTGCGGATGTGGTGCGTCGGA-TGTCCATTCC--CTCGCC--TCCAGC--GT-----TGGAGGGCGTGCTCCAGACGGATGAGG---
3701
3800
MaPAH1.1 CAAGTTGGAGCAGGGGCAGGAGCAGCACGACGGAACAGCAGCAGGAACAGGAACAGGA-ACGAGAGCATGATGTCCAGCTGCGTGCAGCAGCTGAAGGGGA
MaPAH1.2 --AGCTGGCTCAGGCATCGAGCAAGGCGCTGGCGCTTCAG--GGATGGGACACAGCAGATTTGAGCAGAGAG---AGCAGTGTTCAG-GCCAAGAGTGA
3801
3900
MaPAH1.1 GCAGCTTGCTTACACTCGAGAGTACGCGGAAGAAGAAGCCGCTGCTGGATATCTGGCGGAGGACCATGAACTCGGAG-ACGATGAACAGGATGAAGGAGA
MaPAH1.2 TGTGATGGACGACCTTGTGGCGGTCAAGGAGGAAGAGGAGGACGAGACCGATGAG-CAGCGGTTGCTGGATGCAGCGTATGTGGATGAGTATGT-GGATG
3901
4000
MaPAH1.1 AGGAGCAGATGGATATGTTGGTTATTCTGGACAAGAGGATGAAGGTCTGGAAGAAGATCAGCTCGAGGGTGAGGAAGACGAGGATGAGGATGACGATGAT
MaPAH1.2 AGGAG--GATG---AGGACGGATATGATGGATATGACGAGCAGGGT------GAGGATCAGATGGACGAGGAGGATGAGGAGGACGAGTATCTCGATGAG
4001
4033
MaPAH1.1 GTAGAGCTCAACATTGACGCTCCGTTCCTATGA
MaPAH1.2 ATTGAGGAGACTCTCGAGGAGCCGTTCCTGTAG

Figure 7


1 100
PAH1-1 MQSVGSFFSTVSRFYNELNPATLSGAIDVVVVEQADGELACSPFHVRFGKLSILRPQEKVVEVTVNGRVVDFPMKVGDAGEAFFVFETEQDVPEEFATSP
PAH1-2 MYSVGNFFSTVTKFYNEINPATLSGAIDIIVVQQANGDLACSPFHVRFGKLSVLRPQEKVVEVRVNGEVIAFPMKVGDAGEAFFVLETDDYVPDEFATSP
101 200
PAH1-1 LAGPNTDKVEEDIDYLDLAEGHSTVTYPPDDIVLDAGYVSAHSGHGSEFEEDERADLSPEFDKKPDYASAVKYGGTNGQGRHLGSANEATTSVHAFMERQ
PAH1-2 IAGPSDEADLAPVDYFDLNGHPHGSQDQKRRQHQQQQVLEGMSGQYPQGTEDDAPLDNGYVSAASGHGSAFEESLKDDS----DHESVFSATSPGSAERI
201 300
PAH1-1 VQRWSLTMSLPPSPVLKSRDIMENFQPIDSAGPFDNSREDSGRLLAPETIAVSNGGSSGSLFHPKEGMIMDMTGYKTEDSDLNSDASDEHDVGMAGALNG
PAH1-2 AADSNTKDTALDLPGSFGPTVVTNTIKNKDSINFPVDAIFPTVAHEEQDMALIKDQQGSRSSRRRSEVLFDMTGYKTDSCSDSSDDEDGLPRGILSDSER
301 400
PAH1-1 RHRRKRAARRKRRGPVHGVNSQDNLATETPSITAHVLSSLDPRLPLRPTARPALRPKANNGLGTLPNRRSSSMPNLKDFVGENNSLSPSVPAIMRRFPSK
PAH1-2 HGRSTRKKFRRSKSHLSMEQRHQLLEDIKQGAFLKPEESLANTQIERQTSRASRKTKRASIPSAWQGRRNRKRANSMPAIGEPDLAFPAYVARRP------
401 500
PAH1-1 TLNSKFSARSDIKDGTSSSSSVASSPPPSVANQQSPKNRHHHHHHHHKEHTEGSHPRRHSHKPSQQVQVKKPPPRSNPAVNALSDTELEYQTPRTTAATQE
PAH1-2 ----------NHRRDAQANQTDVAMDDKPKPKRTARPSVMSDTEMEYESNNVPASTQG
501 600
PAH1-1 SEWSWGWGSLPVKNDGLGTGEADHKEHHSSHPSIDIPAPRKPVLNEMETDGTVYRLAISLCPGDEFGKDLEASEALFATNQVSFDEFAKDPLKTLNNKNL
PAH1-2 KEWTWGWGTLPVKQDNP---DEEDEIKEQITEEKAPEVPVEIEAKEFQMGSTKCRVALSLCGEDDFGKDIVASHKAFQRAQLTFEAFSKDPAAILADKRL
601 700
PAH1-1 VCLINDRYFTWTAAGPYLSSLMLFRKPLSDETLHQLSAKDSRHLSDRLAVQDEPPTRFGALSRWLRGSQTSSQLSAMEQGQRQRTPSTNDALQPAQLEES
PAH1-2 VCYMDGRFYSWSNAVPQLAALLFFHQPLSDAAS----ALDLKDQKAHAAEDRPSATRFGTISRWFR----------KAPAGSASPSIADMASA
701 800
PAH1-1 QALQSVKVESIKHTSGSHSSLLRAPKPMTRSTSLPIDEGIAGSISDEYAGSSPPTHSALK---SSRRYAKTLRLTSEQLKSLNLKKGANTLTFSVTSSYQ
PAH1-2 SSTTLAGGETAAVAVGSDDDEPLHNKALRSKSLPPLETGRTDDHSQSHVAVPALSEKAADGVPDQKRYAKTLRLTSEQLQSLGLKKGANTVSFSVTSSYQ
801 900
PAH1-1 GKAVCSAKLFLWDHDYQVVISDIDGTITKSDALGHIFTMAGKDWTHSGVAKLYTDIVNNGYHILYLTSRAIGQADYTRKYLKNVEQNNYQLPDGPVIMSP
PAH1-2 GTATCVAKIFLWDYDSQVVISDIDGTITKSDALGHIFAMAGRDWTHLGVAKLFTDIRSNGYHILYLTSRAIGQADYTRKYLQKVEQNSYQLPDGPVIMSP
901 1000
PAH1-1 DRLMTAFHREVIMRKPEEFKMACLRDIRRLFGDRNPFYAGFGNRITDALSYRSVNVPSSRIFTIDSGGEVKLELLSSYKSSYLALNDLVNEIFPGKRQAP
PAH1-2 DRLFSAFHREVIIRKPEVFKMACLRDVKKLFGDRNPFYAGFGNRITDALSYRSVNVPPSRIFTIDSYGEVKLELLSAFKSSYLALNDLVNEIFPGQRVAP
1001 1100
PAH1-1 EFNDWNFWRAPLPDIELPVAPSHQYAPTAVPGEYNAQGYSAGPGRLGVIRSLTSSLTSAGPLKTRTAIPIFTSN-SPPPPNSYPSAMKPHAPHQSQPASS
PAH1-2 EFNDWNFWKSDLPRIDLPDLPIPNNNYTSGSSTSLLSSTTSVAKKVASLTSSSSSSNLLQPTSPTSPTGDFKNKRLSNDRNTYAGVLSGRQDTWTSDDEY
1101 1200
PAH1-1 SPQPP-ASAPSGLQIADRTRRLSLSLMRYSSHSAPTSAPVLRTLTDSSEPNVGIDSGDAGALSEGNQAGLEPNRSPHLGSNTDGVFPLDVPVVKRKASGF
PAH1-2 QDQQQRLIAGDSAPSTPGSELKAGQELKEDARKARSGSPSMLSALVPSRLIRAVRSGSISSQTNPVPSSMRSSVTPHSPEMKGIIGSLPSPVSSFESGAD
1201 1300
PAH1-1 SVSPPQLASRLSETVMPFLRRRASKLEQGQEQQQEQQQEQEQEREHDVQLGAAAEGEQLAYTREYGEEEAAAGYLAEDHELGEDEEDEGEGADGYVGYSG
PAH1-2 VVRRMSIPSPPPLEGLLQTDEEVAQASSKALALQGSDTADLS-RESSVQAKSDVMDDLVAVKEEEEDETDQQRLLDAAYVDEYVDEEDEEGYDGYDEQG-
1301 1333
PAH1-1 EEDEGLEEDQLEGEEDEDEDDDDVELNIDAPFL
PAH1-2 -------EDEMDEEDEEDEYLDEIEETLEEPFL

PHOSPHATIDIC ACID PHOSPHATASE GENE AND USE THEREOF

TECHNICAL FIELD

The present invention relates to a novel phosphatidic acid phosphatase gene and use thereof.

BACKGROUND ART

Fatty acids containing two or more unsaturated bonds are collectively referred to as polyunsaturated fatty acids (PUFAs) and are known to include arachidonic acid, dihomo-γ-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, etc. Some of these polyunsaturated fatty acids cannot be synthesized in the animal body, and such polyunsaturated fatty acids need to be ingested from foods as essential fatty acids. The polyunsaturated fatty acids are widely distributed. For example, arachidonic acid is isolated from lipids extracted from suprarenal gland and liver of animals. However, the amounts of these polyunsaturated fatty acids contained in animal organs are small, and the polyunsaturated fatty acids extracted and isolated from animal organs only are insufficient for a large amount of supply thereof. Thus, microbial techniques have been developed for obtaining polyunsaturated fatty acids by culturing various microorganisms. In particular, microorganisms in the genera *Mortierella* are known to produce lipids containing polyunsaturated fatty acids such as arachidonic acid.

Other attempts have also been made to produce polyunsaturated fatty acids in plants. Polyunsaturated fatty acids are known to constitute reserve lipids such as triacylglycerol (also referred to as triglyceride or TG) and accumulate within microorganism cells or plant seeds.

Triacylglycerol as a reserve lipid is generated in the body as follows: An acyl group is introduced into glycerol-3-phosphate by glycerol-3-phosphate acyltransferase to generate lysophosphatidic acid. An acyl group is introduced into the lysophosphatidic acid by lysophosphate acyltransferase to generate phosphatidic acid. The phosphatidic acid is dephosphorylated by phosphatidic acid phosphatase to generate diacylglycerol. An acyl group is introduced into the diacylglycerol by diacylglycerol acyltransferase to generate triacylglycerol.

In this pathway, phosphatidic acid (hereinafter, also referred to as "PA" or 1,2-diacyl-sn-glycerol-3-phosphate) is a precursor of triacylglycerol and is also a biosynthetic precursor of diacyl glycerophospholipid. In yeast cells, CDP diacylglycerol (CDP-DG) is synthesized from PA and cytidine 5'-triphosphate (CTP), by phosphatidate cytidyltransferase, and is biosynthesized into various phospholipids.

As described above, the reaction of biosynthesizing diacylglycerol (hereinafter, also referred to as "DG") through dephosphorylation of PA is known to be catalyzed by phosphatidic acid phosphatase (E.C. 3.1.3.4, hereinafter, also referred to as "PAP"). This PAP is known to be present in all organisms from bacteria to vertebrates.

Yeast (*Saccharomyces cerevisiae*), which is a fungus, has two types of PAPs (Non-Patent Literatures 1, 2, and 7). One is a Mg^{2+}-dependent PAP (PAP1), and the other is a Mg^{2+}-independent PAP (PAP2). A PAH1 gene is known as a gene encoding PAP1 (Non-Patent Literatures 3 to 5). A pah1Δ variant also shows a PAP1 activity, which suggests there are other genes exhibiting the PAP1 activity. In the pah1Δ variant, the nuclear membrane and the ER membrane are abnormally dilated, and expression of important genes for biosynthesis of phospholipids is abnormally enhanced (Non-Patent Literature 6).

As genes encoding PAP2, a DPP1 gene and a LPP1 gene are known and exhibit most PAP2 activities in yeast. The enzymes encoded by these genes have broad substrate specificity and act also on, for example, diacylglycerol pyrophosphate (DGPP), lysophosphatidic acid, sphingoid base phosphate, and isoprenoid phosphate to dephosphorylate them.

A lipid-producing fungus, *Mortierella alpina*, is known to have a MaPAP1 gene, which is a Mg^{2+}-independent PAP2 homolog (Patent Literature 1).

Existance of gene homologs that probably encode PAP1 family enzymes or PAP2 family enzymes in other bacteria is known in the art, but their functions have not been elucidated.

CITATION LIST

Patent Literature

Patent Literature 1: International Publication No. WO2009/008466

Non-Patent Literature

Non-Patent Literature 1: Biochem. Biophys. Acta, 1348, 45-55, 1997
Non-Patent Literature 2: Trends Biochem. Sci., 31(12), 694-699, 2006
Non-Patent Literature 3: EMBO J., 24, 1931-1941, 2005
Non-Patent Literature 4: J. Biol. Chem., 281(14), 9210-9218, 2006
Non-Patent Literature 5: J. Biol. Chem., 281(45), 34537-34548, 2006
Non-Patent Literature 6: J. Biol. Chem., 282(51), 37026-37035, 2007
Non-Patent Literature 7: J. Biol. Chem., 284(5), 2593-2597, 2009

SUMMARY OF INVENTION

Technical Problem

Most of the PAP genes previously reported, however, have not been investigated for that these genes introduced into host cells and expressed therein can vary the proportion of fatty acids in the fatty acid composition produced by the host cells. There is a demand for identification of a novel gene that can produce fat having an intended composition of fatty acids or an increase in content of an intended fatty acid by introducing the gene into a host cell or expressing the gene.

It is an object of the present invention to provide a protein or a nucleic acid that allows host cells to produce fat having an intended composition of fatty acids or an increase in content of an intended fatty acid by expressing the protein in the host cells or introducing the nucleic acid into the host cells.

Solution to Problem

The present inventors have diligently studied to solve the above-mentioned problems. That is, the inventors have analyzed the genome of lipid-producing fungus, *Mortierella alpina*, and extracted sequences having homology to known

Mg^{2+}-dependent phosphatidic acid phosphatase (PAP1) genes from the genome. Further, cloning of the full-length cDNA through cDNA library screening or PCR were conducted to obtain the entire open reading frame (ORF) encoding PAP, and the gene were introduced into host cells having high proliferative ability, such as yeast. As a result, the inventors have found that the protein encoded by the cloned cDNA has a phosphatidic acid phosphatase activity and that introduction of the cDNA to yeast enhances the production of reserve lipids, triacylglycerol, in the yeast. Thus, cloning of a gene related to a novel phosphatidic acid phosphatase (PAP) has been successfully achieved, and the present invention has been accomplished. That is, the present invention is as follows.

(1) A nucleic acid according to any one of (a) to (g) below:

(a) a nucleic acid comprising a nucleotide sequence encoding a protein that consists of an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and has a phosphatidic acid phosphatase activity;

(b) a nucleic acid comprising a nucleotide sequence that is hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 under stringent conditions and encodes a protein having a phosphatidic acid phosphatase activity;

(c) a nucleic acid comprising a nucleotide sequence which consists of a nucleotide sequence having an identity of 70% or more with the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 and encodes a protein having a phosphatidic acid phosphatase activity;

(d) a nucleic acid comprising a nucleotide sequence encoding a protein that consists of an amino acid sequence having an identity of 70% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and has a phosphatidic acid phosphatase activity;

(e) a nucleic acid comprising a nucleotide sequence that is hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to a nucleotide sequence encoding a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 under stringent conditions and encodes a protein having a phosphatidic acid phosphatase activity;

(f) a nucleic acid comprising a nucleotide sequence that is hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 under stringent conditions and includes an exon encoding a protein having a phosphatidic acid phosphatase activity; and

(g) a nucleic acid comprising a nucleotide sequence which consists of a nucleotide sequence having an identity of 70% or more with the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 and includes an exon encoding a protein having a phosphatidic acid phosphatase activity.

(2) The nucleic acid according to aspect (1), wherein the nucleic acid is any one of (a) to (g) below:

(a) a nucleic acid comprising a nucleotide sequence encoding a protein that consists of an amino acid sequence having deletion, substitution, or addition of 1 to 130 amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and has a phosphatidic acid phosphatase activity;

(b) a nucleic acid comprising a nucleotide sequence that is hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 under conditions of 2×SSC at 50° C. and encodes a protein having a phosphatidic acid phosphatase activity;

(c) a nucleic acid comprising a nucleotide sequence which consists of a nucleotide sequence having an identity of 90% or more with the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 and encodes a protein having a phosphatidic acid phosphatase activity;

(d) a nucleic acid comprising a nucleotide sequence encoding a protein that consists of an amino acid sequence having an identity of 90% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and has a phosphatidic acid phosphatase activity;

(e) a nucleic acid comprising a nucleotide sequence that is hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to a nucleotide sequence encoding a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 under conditions of 2×SSC at 50° C. and encodes a protein having a phosphatidic acid phosphatase activity;

(f) a nucleic acid comprising a nucleotide sequence that is hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 under conditions of 2×SSC at 50° C. and includes an exon encoding a protein having a phosphatidic acid phosphatase activity; and

(g) a nucleic acid comprising a nucleotide sequence which consists of a nucleotide sequence having an identity of 90% or more with the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 and includes an exon encoding a protein having a phosphatidic acid phosphatase activity.

(3) A nucleic acid according to any one of (a) to (d) below:

(a) a nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 or a fragment thereof;

(b) a nucleic acid comprising a nucleotide sequence encoding a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 or a fragment thereof;

(c) a nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO: 4 or SEQ ID NO: 9 or a fragment thereof; and

(d) a nucleic acid comprising the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 or a fragment thereof.

(4) A nucleic acid according to any one of (a) to (g) below:

(a) a nucleic acid comprising a nucleotide sequence encoding a protein that consists of an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and has an activity that enhances generation of diacylglycerol (DG) and/or triglyceride (TG) from phosphatidic acid (PA) in a PAH1-deficient yeast strain;

(b) a nucleic acid comprising a nucleotide sequence that is hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 under stringent conditions and encodes a protein having an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain;

(c) a nucleic acid comprising a nucleotide sequence which consists of a nucleotide sequence having an identity of 70% or more with the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 and encodes a protein having an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain;

(d) a nucleic acid comprising a nucleotide sequence encoding a protein that consists of an amino acid sequence having an identity of 70% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and has an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain;

(e) a nucleic acid comprising a nucleotide sequence that is hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to a nucleotide sequence encoding a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 under stringent conditions and encodes a protein having an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain;

(f) a nucleic acid comprising a nucleotide sequence that is hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 under stringent conditions and includes an exon encoding a protein having an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain; and

(g) a nucleic acid comprising a nucleotide sequence which consists of a nucleotide sequence having an identity of 70% or more with the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 and includes an exon encoding a protein having an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain.

(5) The nucleic acid according to aspect (4), wherein the nucleic acid is any one of (a) to (g) below:

(a) a nucleic acid comprising a nucleotide sequence that encoding a protein that consists of an amino acid sequence having deletion, substitution, or addition of 1 to 130 amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and has an activity that enhances generation of diacylglycerol (DG) and/or triglyceride (TG) from phosphatidic acid (PA) in a PAH1-deficient yeast strain;

(b) a nucleic acid comprising a nucleotide sequence that is hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 under conditions of 2×SSC at 50° C. and encodes a protein having an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain;

(c) a nucleic acid comprising a nucleotide sequence which consists of a nucleotide sequence having an identity of 90% or more with the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 and encodes a protein having an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain;

(d) a nucleic acid comprising a nucleotide sequence encoding a protein that consists of an amino acid sequence having an identity of 90% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and has an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain;

(e) a nucleic acid comprising a nucleotide sequence that is hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to a nucleotide sequence encoding a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 under conditions of 2×SSC at 50° C. and encodes a protein having an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain;

(f) a nucleic acid comprising a nucleotide sequence that is hybridizable with a nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 under conditions of 2×SSC at 50° C. and includes an exon encoding a protein having an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain; and

(g) a nucleic acid comprising a nucleotide sequence which consists of a nucleotide sequence having an identity of 90% or more with the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 and includes an exon encoding a protein having an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain.

(6) A protein according to (a) or (b) below:

(a) a protein consisting of an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and having a phosphatidic acid phosphatase activity; and

(b) a protein consisting of an amino acid sequence having an identity of 70% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and having a phosphatidic acid phosphatase activity.

(7) A protein according to (a) or (b) below:

(a) a protein consisting of an amino acid sequence having deletion, substitution, or addition of 1 to 130 amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and having a phosphatidic acid phosphatase activity; and

(b) a protein consisting of an amino acid sequence having an identity of 90% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and having a phosphatidic acid phosphatase activity.

(8) A protein according to (a) or (b) below:

(a) a protein consisting of an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and having an activity that enhances generation of diacylglycerol (DG) and/or triglyceride (TG) from phosphatidic acid (PA) in a PAH1-deficient yeast strain; and

(b) a protein consisting of an amino acid sequence having an identity of 70% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and having an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain.

(9) A protein according to (a) or (b) below:

(a) a protein consisting of an amino acid sequence having deletion, substitution, or addition of 1 to 130 amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and having an activity that enhances generation of diacylglycerol (DG) and/or triglyceride (TG) from phosphatidic acid (PA) in a PAH1-deficient yeast strain; and

(b) a protein consisting of an amino acid sequence having an identity of 90% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and having an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain.

(10) A protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7.

(11) A recombinant vector comprising a nucleic acid according to any one of aspects (1) to (5).

(12) A transformant transformed with the recombinant vector according to aspect (11).

(13) A fatty acid composition comprising a fatty acid or a lipid obtained by culturing the transformant according to aspect (12).

(14) A method for producing a fatty acid composition, chyaracterized by collecting a fatty acid or a lipid from a culture obtained by culturing the transformant according to aspect (12).

(15) A food comprising the fatty acid composition according to aspect (13).

Advantageous Effects of Invention

The PAP of the present invention can enhance the ability of producing fatty acids and reserve lipids in cells to which PAP has been introduced, and preferably can enhance the productivity of polyunsaturated fatty acids in microorganisms or plants.

The PAP of the present invention is expected to produce fatty acids in a host cell, the fatty acids having a composition different from that of fatty acids produced in a host cell to which PAP is not introduced. This can provide lipids having intended characteristics and effects and is therefore useful in application to, for example, foods, cosmetics, pharmaceuticals, and soap.

BRIEF DESCRIPTION OF DRAWINGS

FIG. **1-1** shows a comparison between a genomic sequence (SEQ ID NO: 5) and an ORF (SEQ ID NO: 1) of a MaPAH1.1 derived from *M. alpina* strain 1S-4.

FIG. **1-2** is the continuation of FIG. **1-1**.

FIG. **1-3** is the continuation of FIG. **1-2**.

FIG. **1-4** is the continuation of FIG. **1-3**.

FIG. **2-1** shows a comparison between genomic sequence (SEQ ID NO: 10) and an ORF (SEQ ID NO: 6) of a MaPAH1.2 derived from *M. alpina* strain 1S-4.

FIG. **2-2** is a continuation of FIG. **2-1**.

FIG. **2-3** is a continuation of FIG. **2-2**.

FIG. **2-4** is a continuation of FIG. **2-3**.

FIG. **3-1** shows the cDNA (SEQ ID NO: 4) of MaPAH1.1 derived from *M. alpina* strain 1S-4 and an amino acid sequence (SEQ ID NO: 2) deduced therefrom.

FIG. **3-2** is a continuation of FIG. **3-1**.

FIG. **3-3** is a continuation of FIG. **3-2**.

FIG. **4-1** shows the cDNA (SEQ ID NO: 9) of MaPAH1.2 derived from *M. alpina* strain 1S-4 and an amino acid sequence (SEQ ID NO: 7) deduced therefrom.

FIG. **4-2** is a continuation of FIG. **4-1**.

FIG. **5-1** shows a comparison of a deduced amino acid sequence (SEQ ID NO: 2) of MaPAH1.1 and a deduced amino acid sequence (SEQ ID NO: 7) of MaPAH1.2 derived from *M. alpina* strain 1S-4 with phosphatidic acid phosphatases of a PAP1 family, a ScPAH1 protein (SEQ ID NO: 19) derived from yeast, *Saccharomyces cerevisiae*, and lipin amino acid sequence (SEQ ID NO: 20) derived from a mouse. In phosphatidic acid phosphatases of a PAP1 family, the N-terminal region is well conserved and is referred to as lipin, N-terminal conserved region (pfam04571). Also in MaPAH1.1 and MaPAH1.2, the N-terminal region is well conserved. In this sequence, the glycine residue indicated by * (corresponding to the 80th amino acid of SEQ ID NO: 2 and the 80th amino acid of SEQ ID NO: 7) is known to be essential for PAP activity. The sequence indicated by a double underline (corresponding to the 819th to 823rd amino acids of SEQ ID NO: 2 and 737th to 741st amino acids of SEQ ID NO: 7) is a DXDX(T/V) motif present in a haloacid dehalogenase (HAD)-like domain. This motif is also conserved in MaPAH1.1 and MaPAH1.2. The sequences upstream and downstream of the motif are also conserved.

FIG. **5-2** is a continuation of FIG. **5-1**.

FIG. **6-1** shows a comparison of a CDS sequence (SEQ ID NO: 3) of MaPAH1.1 and a CDS sequence (SEQ ID NO: 8) of MaPAH1.2 derived from *M. alpina* strain 1S-4.

FIG. **6-2** is a continuation of FIG. **6-1**.

FIG. **6-3** is a continuation of FIG. **6-2**.

FIG. **7** shows a comparison of a deduced amino acid sequence (SEQ ID NO: 2) of MaPAH1.1 with a deduced amino acid sequence (SEQ ID NO: 7) of MaPAH1.2 derived from *M. alpina* strain 1S-4.

DESCRIPTION OF EMBODIMENTS

The present invention relates to a novel phosphatidic acid phosphatase gene derived from genus *Mortierella*, wherein the phosphatidic acid phosphatase dephosphorylates phosphatidic acid to generate diacylglycerol.

The phosphatidic acid phosphatase of the present invention is an enzyme that catalyzes a reaction of generating diacylglycerol by dephosphorylation of phosphatidic acid. The substrate of PAP of the present invention is usually phosphatidic acid, but is not limited thereto.

Nucleic Acid Encoding Phosphatidic Acid Phosphatase of the Present Invention

Phosphatidic acid phosphatase (PAP) of the present invention encompasses MaPAH1.1 and MaPAH1.2. The correspondences between cDNA, CDS, and ORF encoding MaPAH1.1 and MaPAH1.2, as well as a deduced amino acid sequence are summarized in Table 1.

TABLE 1

	MaPAH1.1		MaPAH1.2	
	SEQ ID NO	Corresponding region in SEQ ID NO: 4	SEQ ID NO	Corresponding region in SEQ ID NO: 9
cDNA	SEQ ID NO: 4	*****	SEQ ID NO: 9	*****
CDS	SEQ ID NO: 3	Positions 1 to 3985	SEQ ID NO: 8	Positions 72 to 3791
ORF	SEQ ID NO: 1	Positions 1 to 3982	SEQ ID NO: 6	Positions 72 to 3788
Amino acid sequence	SEQ ID NO: 2	*****	SEQ ID NO: 7	*****

Sequences related to MaPAH1.1 of the present invention include SEQ ID NO: 2, which is the amino acid sequence of MaPAH1.1; SEQ ID NO: 1, which shows the sequence of the ORF region of MaPAH1.1; SEQ ID NO: 3, which shows the sequence of the CDS region of MaPAH1.1; and SEQ ID NO: 4, which is the nucleotide sequence of cDNA for MaPAH1.1. Among them, SEQ ID NO: 3 corresponds to the nucleotides 1 to 3985 of SEQ ID NO: 4, while SEQ ID NO: 1 corresponds to the nucleotides 1 to 3982 of SEQ ID NO: 4 and the nucleotides 1 to 3982 of SEQ ID NO: 3. SEQ ID NO: 5 is a genomic nucleotide sequence encoding MaPAH1.1 of the present invention. The genomic sequence

of SEQ ID NO: 5 is composed of eleven exons and ten introns. In SEQ ID NO: 5, the exon regions correspond to the nucleotides 1 to 182, 370 to 584, 690 to 1435, 1536 to 1856, 1946 to 2192, 2292 to 2403, 2490 to 2763, 2847 to 3077, 3166 to 3555, 3648 to 3862, and 3981 to 5034.

Sequences related to MaPAH1.2 of the present invention include SEQ ID NO: 7, which is the amino acid sequence of MaPAH1.2; SEQ ID NO: 6, which shows the sequence of the ORF region of MaPAH1.2; SEQ ID NO: 8, which shows the sequence of the CDS region of MaPAH1.2; and SEQ ID NO: 9, which is the nucleotide sequence of cDNA for MaPAH1.2. Among them, SEQ ID NO: 8 corresponds to the nucleotides 72-3791 of SEQ ID NO: 9, while SEQ ID NO: 6 corresponds to the nucleotides 72 to 3788 of SEQ ID NO: 9 and the nucleotides 1 to 3717 of SEQ ID NO: 8. SEQ ID NO: 10 is a genomic nucleotide sequence encoding MaPAH1.2 of the present invention. The genomic sequence of SEQ ID NO: 10 consists of eight exons and seven introns. In SEQ ID NO: 10, the exon regions correspond to the nucleotides 1 to 454, 674 to 1006, 1145 to 1390, 1479 to 1583, 1662 to 1804, 1905 to 2143, 2243 to 3409, and 3520 to 4552.

The nucleic acids of the present invention encompass single-stranded and double-stranded DNAs and also complementary RNAs thereof, which may be either naturally occurring or artificially prepared. Examples of DNA include, but not limited to, genomic DNAs, cDNAs corresponding to the genomic DNAs, chemically synthesized DNAs, PCR-amplified DNAs, combinations thereof, and DNA/RNA hybrids.

Preferred embodiments for the nucleic acids of the present invention include (a) nucleic acids comprising the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6, (b) nucleic acids comprising a nucleotide sequence encoding a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7, (c) nucleic acids comprising the nucleotide sequence set forth in SEQ ID NO: 4 or SEQ ID NO: 9, and (d) nucleic acids comprising the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10.

In order to obtain these nucleotide sequences, nucleotide sequence data of ESTs or genomic DNAs from organisms having PAP activity may be used to search a nucleotide sequence encoding a protein having a high identity with known proteins having PAP activity. Preferred organisms having PAP activity are lipid-producing fungi including, but not limited to, *M. alpina*.

For EST analysis, a cDNA library is first prepared. The cDNA library may be prepared by referring to "Molecular Cloning, A Laboratory Manual 3rd ed." (Cold Spring Harbor Press (2001)). Alternatively, a commercially available cDNA library preparation kit may be used. Examples of a method of preparing a cDNA library suitable for the present invention are as follows. That is, an appropriate strain of *M. alpina*, a lipid-producing fungus, is inoculated into an appropriate medium and pre-cultured for an appropriate period. Culture conditions suitable for this pre-culture are, for example, a medium composition of 1.8% glucose, 1% yeast extract, and pH 6.0, a culture period of 3 to 4 days, and a culture temperature of 28° C. The pre-cultured product is then subjected to main culture under appropriate conditions. A medium composition suitable for the main culture is, for example, 1.8% glucose, 1% soybean powder, 0.1% olive oil, 0.01% Adekanol, 0.3% KH_2PO_4, 0.1% Na_2SO_4, 0.05% $CaCl_2.2H_2O$, and 0.05% $MgCl_2.6H_2O$, and pH 6.0. Culture conditions suitable for the main culture are, for example, aeration and agitation culture at 300 rpm, 1 vvm, and 26° C. for 8 days. An appropriate amount of glucose may be added during culture. The cultured product is sampled at appropriate time points during the main culture, from which the cells are collected to prepare total RNA. The total RNA may be prepared by any known method such as a guanidine hydrochloride/CsCl method. From the resulting total RNA, poly(A)$^+$ RNA can be purified using a commercially available kit, and a cDNA library can be prepared using a commercially available kit. The nucleotide sequence of any clone from the prepared cDNA library is determined using primers that are designed on a vector to allow determination of the nucleotide sequence of an insert. As a result, ESTs can be obtained. For example, when a ZAP-cDNA GigapackIII Gold Cloning Kit (Stratagene Inc.) is used for preparing a cDNA library, directional cloning is possible.

In analysis of genomic DNA, cells of an organism having PAP activity are cultured, and genomic DNA is prepared from the cells. The nucleotide sequence of the resulting genomic DNA is determined, and the determined nucleotide sequence is assembled. From the finally obtained supercontig sequence, a sequence encoding an amino acid sequence having a high homology to the amino acid sequence of a known protein having PAP activity is searched. From the supercontig sequence giving a hit as that encoding such an amino acid sequence, primers are prepared. PCR is performed using the cDNA library as a template, and the resulting DNA fragment is inserted into a plasmid for cloning. PCR is performed using the cloned plasmid as a template and the above-mentioned primers to prepare a probe. The cDNA library is screened using the resulting probe.

A homology search of deduced amino acid sequences of MaPAH1.1 and MaPAH1.2 of the present invention was performed against amino acid sequences registered in GenBank with BLASTp program. These deduced amino acid sequences of MaPAH1.1 and MaPAH1.2 give a hit with nuclear elongation and deformation protein 1 putative protein (AAW42851) derived from *Cryptococcus neoformans* var. *neoformans* JEC21 with the highest scores, and the identities are 25.9% and 26.6%, respectively. The deduced amino acid sequences of MaPAH1.1 and MaPAH1.2 of the present invention have identities of 22.7% and 22.5%, respectively, with the amino acid sequence of *S. cerevisiae*-derived PAH1 protein (throughout the specification, also referred to as PAH1 of yeast or ScPAH1), which has been functionally analyzed, among PAP1 homologs of fungi.

The present invention also encompasses nucleic acids functionally equivalent to a nucleic acid including the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 (hereinafter also referred to as "the nucleotide sequence of the present invention") or a nucleotide sequence encoding a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 (hereinafter also referred to as "the amino acid sequence of the present invention"). The term "functionally equivalent" refers to that a protein encoded by the nucleotide sequence of the present invention and a protein consisting of the amino acid sequence of the present invention have a phosphatidic acid phosphatase (PAP) activity. In addition, the term "functionally equivalent" includes the activity that enhances generation of diacylglycerol (DG) and/or triglyceride (TG) from phosphatidic acid (PA) in a PAH1-deficient yeast strain when a protein encoded by the nucleotide sequence of the present invention or a protein consisting of the amino acid sequence of the present invention is expressed. The PAP activity of the protein of the present invention and the activity that enhances generation of DG and/or TG from PA

in a PAH1-deficient yeast strain may be Mg^{2+}-dependent or Mg^{2+}-independent. The activity of the protein of the present invention is preferably Mg^{2+}-dependent.

Such nucleic acids that are functionally equivalent to the nucleic acids of the present invention include nucleic acids comprising nucleotide sequences shown in any one of (a) to (g) below. It should be noted that in the descriptions of the nucleotide sequences listed below, the term "the activity of the present invention" refers to "the PAP activity and/or the activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain".

(a) A nucleic acid comprising a nucleotide sequence encoding a protein that consists of an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and has the activity of the present invention

The nucleotide sequence contained in the nucleic acid of the present invention encompasses nucleotide sequences encoding a protein that consists of an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and has the activity of the present invention.

Specifically, the nucleotide sequence contained in the nucleic acid of the present invention is a nucleotide sequence encoding a protein having the above-described activity of the present invention and consisting of:

(i) an amino acid sequence having deletion of one or more (preferably one to several (e.g., 1 to 400, 1 to 200, 1 to 130, 1 to 100, 1 to 75, 1 to 50, 1 to 30, 1 to 25, 1 to 20, or 1 to 15, more preferably 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1)) amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7;

(ii) an amino acid sequence having substitution of one or more (preferably one to several (e.g., 1 to 400, 1 to 200, 1 to 130, 1 to 100, 1 to 75, 1 to 50, 1 to 30, 1 to 25, 1 to 20, or 1 to 15, more preferably 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1)) amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7;

(iii) an amino acid sequence having addition of one or more (preferably one to several (e.g., 1 to 400, 1 to 200, 1 to 130, 1 to 100, 1 to 75, 1 to 50, 1 to 30, 1 to 25, 1 to 20, or 1 to 15, more preferably 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1)) amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7; or

(iv) an amino acid sequence in any combination of (i) to (iii) above.

Among the above, substitution is preferably conservative, which means replacement of a certain amino acid residue by another residue having similar physical and chemical characteristics. It may be any substitution that does not substantially alter the structural characteristics of the original sequence. For example, any substitution is possible as long as the substituted amino acids do not disrupt the helix of the original sequence or do not disrupt any other type of secondary structure characterizing the original sequence.

Conservative substitution is generally introduced by synthesis with a biological system or chemical peptide synthesis, preferably by chemical peptide synthesis. In such a case, substituents may include an unnatural amino acid residue, a peptidomimetic, or a reversed or inverted form where an unsubstituted region is reversed or inverted in the amino acid sequence.

Unlimited examples of the mutually substitutable amino acid residues are classified and listed below:

Group A: leucine, isoleucine, norleucine, valine, norvaline, alanine, 2-aminobutanoic acid, methionine, O-methylserine, t-butylglycine, t-butylalanine, and cyclohexylalanine;

Group B: aspartic acid, glutamic acid, isoaspartic acid, isoglutamic acid, 2-aminoadipic acid, and 2-aminosuberic acid;

Group C: asparagine and glutamine;

Group D: lysine, arginine, ornithine, 2,4-diaminobutanoic acid, and 2,3-diaminopropionic acid;

Group E: proline, 3-hydroxyproline, and 4-hydroxyproline;

Group F: serine, threonine, and homoserine; and

Group G: phenylalanine and tyrosine.

In non-conservative substitution, replacement of a member of one of the above classes by a member from another class is possible. In such a case, in order to maintain the biological function of the protein of the present invention, the hydropathic indices of amino acids (hydropathic amino acid indices) (Kyte, et al., J. Mol. Biol., 157: 105-131 (1982)) are preferably considered.

In the case of non-conservative substitution, amino acid substitutions can be accomplished on the basis of hydrophilicity.

Note that in either conservative substitution or non-conservative substitution, the amino acid residue corresponding to the 80th amino acid in SEQ ID NO: 2 or SEQ ID NO: 7 is preferably glycine, and the region corresponding to the 819 to 823 amino acids of SEQ ID NO: 2 or the 737 to 741 amino acids of SEQ ID NO: 7 is preferably DXDX (T/V) (X is an arbitrary amino acid).

Throughout the specification and drawings, nucleotides, amino acids, and abbreviations thereof are those according to the IUPAC-IUB Commission on Biochemical Nomenclature or those conventionally used in the art, for example, as described in Immunology—A Synthesis (second edition, edited by E. S. Golub and D. R. Gren, Sinauer Associates, Sunderland, Mass. (1991)). Moreover, amino acids which may have optical isomers are intended to represent their L-isomers, unless otherwise specified.

Stereoisomers such as D-amino acids of the above-mentioned amino acids, unnatural amino acids such as α,α-disubstituted amino acids, N-alkylamino acids, lactic acid, and other unconventional amino acids can also be members constituting the proteins of the present invention.

Note that in the protein notation used throughout the specification, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxy terminal direction, in accordance with standard usage and convention in the art.

Similarly, in general, unless otherwise specified, the left-hand end of single-stranded polynucleotide sequences is the 5'-end and the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5'-direction.

Those skilled in the art would be able to design and prepare appropriate mutants of the proteins described in the specification by using techniques known in the art. For example, a region in the protein molecule suitable for changing the structure without impairing the biological activity of the protein of the present invention can be identified by targeting a region which appears to be less important for the biological activity of the protein. It is also possible to identify residues or regions conserved between similar proteins. Moreover, it is also possible to introduce conservative amino acid substitution into a region that appears to be important for the biological activity or structure of the protein of the present invention, without impair-

ing the biological activity and without adversely affecting the polypeptide structure of the protein.

In particular, in the amino acid sequences of MaPAH1.1 and MaPAH1.2, an amino acid sequence of about 100 amino acids at the N-terminal region, which is referred to as lipin, N-terminal conserved region: pfam04571) in regard of a Mg^{2+}-dependent phosphatidic acid phosphatase (PAP1) family enzyme, is relatively well conserved. Moreover, the amino acid sequences of MaPAH1.1 and MaPAH1.2 each have a "DXDX(T/V) catalytic site motif", which is a conserved motif of a haloacid dehalogenase (HAD)-like protein superfamily enzyme. In FIG. **5**, DIDGT sequences (corresponding to the 819 to 823 residues of SEQ ID NO: 2 and the 737 to 741 residues of SEQ ID NO: 7) indicated with double underlines correspond to these motifs. The mutants of the present invention may be any mutant that conserves the conserved motif and maintains the above-described activity. It has been reported that a variation in this conserved motif site in the PAP1 of yeast loses the PAP activity (J. Biol. Chem., 282 (51): 37026-37035, (2007)).

Those skilled in the art would be able to conduct a so-called structure-function study which identifies residues of a peptide that is important for a biological activity or structure of a protein of the present invention and residues of a peptide similar to the protein, compares the amino acid residues between these two peptides, and thereby predicts which residue in the protein similar to the protein of the present invention is the amino acid residue corresponding to the important amino acid residue for the biological activity or structure. Moreover, it is possible to select a mutant which maintains the biological activity of the protein of the present invention by selecting amino acid substituent chemically similar to the predicted amino acid residue. Likewise, those skilled in the art would also be able to analyze the three-dimensional structure and amino acid sequence of this protein mutant. The analysis results thus obtained can further be used to predict the alignment of amino acid residues involved in the three-dimensional structure of the protein. Though amino acid residues predicted to be on the protein surface may be involved in important interaction with other molecules, those skilled in the art would be able to prepare a mutant which causes no change in these amino acid residues predicted to be on the protein surface, on the basis of analysis results as mentioned above. Moreover, those skilled in the art would also be able to prepare a mutant having a single amino acid substitution for any of the amino acid residues constituting the protein of the present invention. These mutants may be screened by any known assay to collect information about the individual mutants, which in turn allows evaluation of the usefulness of individual amino acid residues constituting the protein of the present invention by comparison of the case where a mutant having substitution of a specific amino acid residue shows a lower biological activity than that of the protein of the present invention, the case where such a mutant shows no biological activity, or where such a mutant produces unsuitable activity that inhibits the biological activity of the protein of the present invention. Moreover, those skilled in the art can readily analyze amino acid substitutions undesirable for mutants of the protein of the present invention based on information collected from such routine experiment alone or in combination with other mutations.

As described above, a protein consisting of an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 can be prepared according to techniques such as site-directed mutagenesis as described in, for example, "Molecular Cloning, A Laboratory Manual 3rd ed." (Cold Spring Harbor Press (2001)); "Current Protocols in Molecular Biology" (John Wiley & Sons (1987-1997); Kunkel, (1985), Proc. Natl. Acad. Sci. USA, 82: 488-92; or Kunkel, (1988), Method Enzymol., 85: 2763-6. Preparation of a mutant with such a mutation including amino acid deletion, substitution, or addition may be accomplished, for example, by known procedures such as a Kunkel method or a Gapped duplex method using a mutation-introducing kit based on site-directed mutagenesis such as a QuikChange™ Site-Directed Mutagenesis Kit (manufactured by Stratagene), a GeneTailor™ Site-Directed Mutagenesis System (manufactured by Invitrogen), or a TaKaRa Site-Directed Mutagenesis System (e.g., Mutan-K, Mutan-Super Express Km; manufactured by Takara Bio Inc.).

Techniques for allowing deletion, substitution, or addition of one or more amino acids in the amino acid sequence of a protein while maintaining its activity include, in addition to site-directed mutagenesis mentioned above, a method of treating a gene with a mutagen and a method selectively cleaving a gene and deleting, substituting or adding a selected nucleotide, and then ligating the gene.

The nucleotide sequence contained in the nucleic acid of the present invention is preferably a nucleotide sequence that encodes a protein consisting of an amino acid sequence having deletion, substitution, or addition of 1 to 130 amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and having PAP activity.

The nucleotide sequence contained in the nucleic acid of the present invention preferably encompasses nucleotide sequences that encode a protein consisting of an amino acid sequence having deletion, substitution, or addition of 1 to 130 amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and having the activity of the present invention.

The number and sites of amino acid mutations or modifications in the protein of the present invention are not limited as long as the PAP activity or the activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain is maintained.

The PAP activity or the activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain can be measured by a known method, for example, see J. Biol. Chem., 273, 14331-14338 (1998).

For example, the "PAP activity" of the present invention may be measured as follows: A crude enzyme solution is prepared by disrupting transformed cells expressing PAP of the present invention, centrifugating the lysate, and collecting the supernatant. The resulting crude enzyme solution may be further subjected to purification of PAP of the present invention. The crude enzyme solution containing PAP of the present invention or purified PAP of the present invention is added to a reaction solution containing 0.5 mM phosphatidic acid, 10 mM 2-mercaptoethanol, and 50 mM Tris-HCl (pH 7.5), followed by reaction at 25° C. to 28° C. for an appropriate time. The reaction is terminated by addition of a mixture of chloroform and methanol, and lipids are extracted. The resulting lipids are fractionated by thin layer chromatography to measure the amount of generated diacylglycerol.

The "activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain" may be measured by, for example, as follows: A PAH1-deficient yeast strain is prepared by disrupting the ScPAH1 gene of yeast (*S. cerevisiae*). The PAH1-deficient yeast strain as a host cell is transformed using a vector containing a nucleic acid encoding PAP of the present invention, and the transformed strain

is cultured. The culture solution is centrifugated to collect the cells. The cells are washed with water and lyophilized. Chloroform and methanol are added to the dried cells, and the cells are disrupted with glass beads to extract lipids. The extracted lipids are fractionated by thin layer chromatography, and the amount of generated DG and/or TG is measured. The PAH1-deficient yeast strain transformed with a vector not containing the nucleic acid encoding PAP of the present invention is used as a control for comparison. If the amount of generated DG and/or TG is increased in a PAH1-deficient yeast strain transformed with a vector containing a nucleic acid encoding PAP of the present invention, the PAP is determined to have "an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain".

(b) A nucleic acid comprising a nucleotide sequence that is hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 under stringent conditions and encodes a protein having the activity of the present invention

The nucleotide sequence contained in the nucleic acid of the present invention encompasses nucleotide sequences that are hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 under stringent conditions and encode a protein having the activity of the present invention.

Such a nucleotide sequence can be prepared from, for example, a cDNA library or a genomic library by a known hybridization technique such as colony hybridization, plaque hybridization, or Southern blotting using a probe produced from an appropriate fragment by a method known to those skilled in the art.

Detailed procedure of the hybridization can be referred to "Molecular Cloning, A Laboratory Manual 3rd ed." (Cold Spring Harbor Press (2001), in particular, Sections 6 and 7), "Current Protocols in Molecular Biology" (John Wiley & Sons (1987-1997), in particular, Sections 6.3 and 6.4), and "DNA Cloning 1: Core Techniques, A Practical Approach 2nd ed." (Oxford University (1995), in particular, Section 2.10 for hybridization conditions).

The strength of hybridization conditions is determined primarily based on hybridization conditions, more preferably based on hybridization conditions and washing conditions. The term "stringent conditions" used throughout the specification is intended to include moderately or highly stringent conditions.

Specifically, examples of the moderately stringent conditions include hybridization conditions of 1×SSC to 6×SSC at 42° C. to 55° C., more preferably 1×SSC to 3×SSC at 45° C. to 50° C., and most preferably 2×SSC at 50° C. In the case of a hybridization solution containing, for example, about 50% formamide, a hybridization temperature of lower than the temperature mentioned above by 5° C. to 15° C. is employed. Washing conditions are, for example, 0.5×SSC to 6×SSC at 40° C. to 60° C. To the hybridization solution and washing solution, 0.05% to 0.2% SDS, preferably about 0.1% SDS, may usually be added.

Highly stringent (high stringent) conditions include hybridization and/or washing at higher temperature and/or lower salt concentration, compared to the moderately stringent conditions. Examples of the hybridization conditions include 0.1×SSC to 2×SSC at 55° C. to 65° C., more preferably 0.1×SSC to 1×SSC at 60° C. to 65° C., and most preferably 0.2×SSC at 63° C. Washing conditions are, for example, 0.2×SSC to 2×SSC at 50° C. to 68° C., and more preferably 0.2×SSC at 60° C. to 65° C.

Examples of the hybridization conditions particularly used in the present invention include, but not limited to, prehybridization in 5×SSC, 1% SDS, 50 mM Tris-HCl (pH 7.5) and 50% formamide at 42° C., overnight incubation at 42° C. in the presence of a probe to form hybrids, and washing in 0.2×SSC, 0.1% SDS at 65° C. for 20 minutes three times.

It is also possible to use a commercially available hybridization kit not using radioactive substance as a probe. Specifically, for example, a DIG nucleic acid detection kit (Roche Diagnostics) or an ECL direct labeling & detection system (manufactured by Amersham) is used for hybridization.

Preferred examples of the nucleotide sequence falling within the present invention include nucleotide sequences that are hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 under conditions of 2×SSC at 50° C. and encode a protein having PAP activity.

(c) A nucleic acid comprising a nucleotide sequence which consists of a nucleotide sequence having an identity of 70% or more with the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 and encodes a protein having the activity of the present invention

The nucleotide sequence contained in the nucleic acid of the present invention encompasses nucleotide sequences which consists of a nucleotide sequence having an identity of at least 70% with the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 and encode a protein having the activity of the present invention.

Preferably, for example, a nucleic acid comprises a nucleotide sequence having an identity of at least 75%, more preferably 80% or more (e.g., 85% or more, more preferably 90% or more, and most preferably 95%, 98%, or 99% or more) with the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 and encoding a protein having the activity of the present invention.

The percent identity between two nucleotide sequences can be determined by visual inspection and mathematical calculation, but is preferably determined by comparing sequence information of two nucleic acids using a computer program. As computer programs for sequence comparison, for example, the BLASTN program (Altschul et al., (1990), J. Mol. Biol., 215: 403-10) version 2.2.7, available via the National Library of Medicine website: www.ncbi.nlm.nih-.gov/blast/bl2seq/bls.html or the WU-BLAST 2.0 algorithm can be used. Standard default parameter settings for WU-BLAST 2.0 are described at the following Internet site: blast.wustl.edu.

(d) A nucleic acid comprising a nucleotide sequence encoding an amino acid sequence having an identity of 70% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and encoding a protein having the activity of the present invention

The nucleotide sequence contained in the nucleic acid of the present invention encompasses nucleotide sequences encoding an amino acid sequence having an identity of 70% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and encoding a protein having the activity of the present invention. The protein encoded by the nucleic acid of the present invention may be a protein having an identity with the amino acid sequence of MaPAH1.1 or MaPAH1.2 as long as the protein is functionally equivalent to the protein having the activity of the present invention.

Specific examples of the protein include amino acid sequences having an identity of 75% or more, preferably 80% or more, more preferably 85% or more, and most preferably 90% or more (e.g., 95% or more, furthermore 98% or more) with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7.

The nucleotide sequence contained in the nucleic acid of the present invention is preferably a nucleotide sequence encoding an amino acid sequence having an identity of 90% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and encoding a protein having the activity of the present invention. More preferably, a nucleotide sequence encoding an amino acid sequence having an identity of 95% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and encoding a protein having the activity of the present invention.

The percent identity between two amino acid sequences can be determined by visual inspection and mathematical calculation or can be determined using a computer program. Examples of such a computer program include BLAST, FASTA (Altschul et al., J. Mol. Biol., 215: 403-410, (1990)) and ClustalW. In particular, various conditions (parameters) for an identity search with the BLAST program are described by Altschul et al. (Nucl. Acids. Res., 25, pp. 3389-3402, 1997) and publicly available via the website of the National Center for Biotechnology Information (NCBI) of USA or the DNA Data Bank of Japan (DDBJ) (BLAST Manual, Altschul et al., NCB/NLM/NIH Bethesda, Md. 20894; Altschul et al.). It is also possible to use a program such as genetic information processing software GENETYX Ver. 7 (Genetyx Corporation), DINASIS Pro (Hitachisoft), or Vector NTI (Infomax) for determination of the percent identity.

A specific alignment scheme for aligning a plurality of amino acid sequences can show matching of sequences also in a specific short region and can therefore detect a region having a very high sequence identity in such a short region even if the full-length sequences have no significant relationship therebetween. In addition, the BLAST algorithm can use the BLOSUM62 amino acid scoring matrix, and the following separation parameters can be used: (A) inclusion of filters to mask a segment of a query sequence having low compositional complexity (as determined by the SEG program of Wootton and Federhen (Computers and Chemistry, 1993); also see Wootton and Federhen, 1996, "Analysis of compositionally biased regions in sequence databases", Methods Enzymol., 266: 554-71) or to mask segments consisting of short-periodicity internal repeats (as determined by the XNU program of Claverie and States (Computers and Chemistry, 1993), and (B) a statistical significance threshold for reporting matches against database sequences, or the expected probability of matches being found merely by chance, according to the statistical model of E-score (Karlin and Altschul, 1990); if the statistical significance ascribed to a match is greater than this E-score threshold, the match will not be reported.

(e) A nucleic acid comprising a nucleotide sequence that is hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to a nucleotide sequence encoding a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 under stringent conditions and encodes a protein having the activity of the present invention

The nucleotide sequence contained in the nucleic acid of the present invention encompasses nucleotide sequences that are hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to a nucleotide sequence encoding a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 under stringent conditions and encode a protein having the activity of the present invention.

The protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and the hybridization conditions are as described above. Examples of the nucleotide sequence contained in the nucleic acid of the present invention include nucleotide sequences that are hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to a nucleotide sequence encoding a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 under stringent conditions and encode a protein having the activity of the present invention.

(f) A nucleic acid comprising a nucleotide sequence that is hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 under stringent conditions and includes an exon encoding a protein having the activity of the present invention

The nucleotide sequences set forth in SEQ ID NO: 5 and SEQ ID NO: 10 are respectively the genomic DNA sequences encoding MaPAH1.1 and MaPAH1.2 of the present invention.

The nucleotide sequence contained in the nucleic acid of the present invention encompasses nucleotide sequences that are hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 under stringent conditions and include an exon encoding a protein having the activity of the present invention.

Such a nucleotide sequence can be prepared by a method known to those skilled in the art from, for example, a genomic library by a known hybridization technique such as colony hybridization, plaque hybridization, or Southern blotting using a probe produced using an appropriate fragment. The hybridization conditions are as described above.

(g) A nucleic acid comprising a nucleotide sequence which consists of a nucleotide sequence having an identity of 70% or more with the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 and includes an exon encoding a protein having the activity of the present invention

The nucleotide sequence contained in the nucleic acid of the present invention encompasses nucleotide sequences which consists of a nucleotide sequence having an identity of at least 70% with the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 and encode a protein having the activity of the present invention. Preferred examples of the nucleotide sequence include those having an identity of at least 75%, more preferably 80% or more (e.g., 85% or more, more preferably 90% or more, and most preferably 95%, 98%, or 99% or more) with the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 and having an exon encoding a protein having the activity of the present invention. The percent identity between two nucleotide sequences can be determined as described above.

The genomic DNA sequence of SEQ ID NO: 5 is composed of eleven exons and ten introns. In SEQ ID NO: 5, the exon regions correspond to nucleotides 1 to 182, 370 to 584, 690 to 1435, 1536 to 1856, 1946 to 2192, 2292 to 2403, 2490 to 2763, 2847 to 3077, 3166 to 3555, 3648 to 3862, and 3981 to 5034. The genomic DNA sequence of SEQ ID NO: 10 is composed of eight exons and seven introns. In SEQ ID NO: 10, the exon regions correspond to nucleotides 1 to 454,

674 to 1006, 1145 to 1390, 1479 to 1583, 1662 to 1804, 1905 to 2143, 2243 to 3409, and 3520 to 4552.

In another embodiment, examples of the nucleotide sequence contained in the nucleic acid of the present invention include nucleotide sequences including intron regions having a nucleotide sequence identity of 100% with the genomic DNA sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 and exon regions having a nucleotide sequence identity of at least 70% or more, more preferably 75% or more, and more preferably 80% or more (e.g., 85% or more, more preferably 90% or more, and most preferably 95%, 98%, or 99% or more) with the sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10, wherein the exon encodes a protein having the activity of the present invention.

In another embodiment, examples of the nucleotide sequence contained in the nucleic acid of the present invention include nucleotide sequences including exon regions having a nucleotide sequence identity of 100% with the genomic DNA sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 and intron regions having a nucleotide sequence identity of at least 70% or more, more preferably 75% or more, and more preferably 80% or more (e.g., 85% or more, more preferably 90% or more, and most preferably 95%, 98%, or 99% or more) with the sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10, wherein the intron regions can be eliminated by splicing, and thereby the exon regions are ligated to encode a protein having the activity of the present invention.

In another embodiment, examples of the nucleotide sequence contained in the nucleic acid of the present invention include nucleotide sequences including intron regions having a nucleotide sequence identity of at least 70% or more, more preferably 75% or more, and more preferably 80% or more (e.g., 85% or more, more preferably 90% or more, and most preferably 95%, 98%, or 99% or more) with the genomic DNA sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 and exon regions having a nucleotide sequence identity of at least 70% or more, more preferably 75% or more, and more preferably 80% or more (e.g., 85% or more, more preferably 90% or more, and most preferably 95% or more, 98% or more, or 99% or more) with the sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10, wherein the intron regions can be eliminated by splicing, and thereby the exon regions are ligated to encode a protein having the activity of the present invention.

The percent identity between two nucleotide sequences can be determined by the method described above.

The nucleic acid of the present invention encompasses nucleic acids each consisting of a nucleotide sequence having deletion, substitution, or addition of one or more nucleotides in the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 and encoding a protein having the activity of the present invention. More specifically, a usable nucleic acid include any one of the following nucleotide sequences:

(i) a nucleotide sequence having deletion of one or more (preferably one to several (e.g., 1 to 1200, 1 to 1000, 1 to 750, 1 to 500, 1 to 400, 1 to 300, 1 to 250, 1 to 200, 1 to 150, 1 to 100, 1 to 50, 1 to 30, 1 to 25, 1 to 20, or 1 to 15, more preferably, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1)) nucleotides in the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6;

(ii) a nucleotide sequence having substitution of one or more (preferably one to several (e.g., 1 to 1200, 1 to 1000, 1 to 750, 1 to 500, 1 to 400, 1 to 300, 1 to 250, 1 to 200, 1 to 150, 1 to 100, 1 to 50, 1 to 30, 1 to 25, 1 to 20, or 1 to 15, more preferably, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1)) nucleotides in the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6;

(iii) a nucleotide sequence having addition of one or more (preferably one to several (e.g., 1 to 1200, 1 to 1000, 1 to 750, 1 to 500, 1 to 400, 1 to 300, 1 to 250, 1 to 200, 1 to 150, 1 to 100, 1 to 50, 1 to 30, 1 to 25, 1 to 20, or 1 to 15, more preferably, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1)) nucleotides in the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6; or

(iv) a nucleotide sequence with any combination of (i) to (iii) above, wherein the nucleotide sequence encodes a protein having the activity of the present invention.

A preferred embodiment of the nucleic acid of the present invention also encompasses nucleic acids comprising a fragment of a nucleotide sequence shown in any one of (a) to (d) below:

(a) the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6;

(b) a nucleotide sequence encoding a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7;

(c) the nucleotide sequence set forth in SEQ ID NO: 4 or SEQ ID NO: 9; and

(d) the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10.

(A) the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6, (b) the nucleotide sequence encoding a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7, and (c) the nucleotide sequence set forth in SEQ ID NO: 4 or SEQ ID NO: 9 are as shown in Table 1. The nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 is also as described above. The fragments of these sequences are ORF, CDS, a biologically active region, a region used as a primer as described later, and a region which may serve as a probe contained in these nucleotide sequences, and may be either naturally occurring or artificially prepared.

The nucleic acid of the present invention encompasses the following nucleic acids.

(1) Nucleic acids shown in any one of (a) to (g) below:

(a) nucleic acids comprising a nucleotide sequence encoding a protein consisting of an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7;

(b) nucleic acids hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 under stringent conditions;

(c) nucleic acids comprising a nucleotide sequence having an identity of 70% or more with the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6;

(d) nucleic acids comprising a nucleotide sequence encoding a protein consisting of an amino acid sequence having an identity of 70% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7;

(e) nucleic acids hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to a nucleotide sequence encoding a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 under stringent conditions;

(f) nucleic acids hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 under stringent conditions; and

(g) nucleic acids comprising a nucleotide sequence having an identity of 70% or more with the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10.

(2) Nucleic acids described in (1) above, shown in any one of (a) to (g) below:

(a) nucleic acids comprising a nucleotide sequence encoding a protein consisting of an amino acid sequence having deletion, substitution, or addition of 1 to 130 amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7;

(b) nucleic acids hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6 under conditions of 2×SSC at 50° C.;

(c) nucleic acids comprising a nucleotide sequence having an identity of 90% or more with the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6;

(d) nucleic acids comprising a nucleotide sequence encoding a protein consisting of an amino acid sequence having an identity of 90% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7;

(e) nucleic acids hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to a nucleotide sequence encoding a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 under conditions of 2×SSC at 50° C.;

(f) nucleic acids hybridizable with a nucleic acid consisting of a nucleotide sequence complementary to the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10 under conditions of 2×SSC at 50° C.; and

(g) nucleic acids comprising a nucleotide sequence having an identity of 90% or more with the nucleotide sequence set forth in SEQ ID NO: 5 or SEQ ID NO: 10.

Phosphatidic Acid Phosphatase of the Present Invention

The protein of the present invention encompasses a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and proteins functionally equivalent to such a protein. These proteins may be either naturally occurring or artificially prepared. The protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 is as described above. The "proteins functionally equivalent" refers to proteins having "the activity of the present invention" described in the "Nucleic acid encoding phosphatidic acid phosphatase of the present invention" above.

In the present invention, examples of the proteins functionally equivalent to a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 include proteins shown in (a) and (b) below:

(a) proteins comsisting of an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and having the activity of the present invention; and

(b) proteins consisting of an amino acid sequence having an identity of 70% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 and having the activity of the present invention.

In the above, the amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7 or the amino acid sequence having an identity of 70% or more with the amino acid sequence set forth in SEQ ID NO: 2 are as described in the "Nucleic acid encoding phosphatidic acid phosphatase of the present invention" above. The "protein having the activity of the present invention" encompasses mutants of proteins encoded by a nucleic acid containing the nucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6; mutated proteins by many types of modification such as deletion, substitution, and addition of one or more amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7; those proteins modified having, for example, modified amino acid side chains; and those proteins fused with other proteins, where these proteins have the PAP activity and/or the activity that enhances generation of diacylglycerol (DG) and/or triglyceride (TG) from phosphatidic acid (PA) in a PAH1-deficient yeast strain.

The protein of the present invention may be artificially prepared. In such a case, the protein can be produced by chemical synthesis such as a Fmoc method (fluorenylmethyloxycarbonyl method) or a tBoc method (t-butyloxycarbonyl method). In addition, peptide synthesizers available from Advanced ChemTech, Perkin Elmer, Pharmacia, Protein Technology Instrument, Synthecell-Vega, PerSeptive, Shimadzu Corporation, or other manufacturers may be used for chemical synthesis.

The protein of the present invention further encompasses the following proteins:

(1) (a) proteins consisting of an amino acid sequence having deletion, substitution, or addition of one or more amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7;

(b) proteins consisting of an amino acid sequence having an identity of 80% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7; and

(2) proteins according to any one of (a) and (b) below:

(a) proteins consisting of an amino acid sequence having deletion, substitution, or addition of 1 to 200 amino acids in the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7; and

(b) proteins consisting of an amino acid sequence having an identity of 90% or more with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 7.

Cloning of Nucleic Acid of the Present Invention

The PAP nucleic acid of the present invention can be cloned by, for example, screening from a cDNA library using an appropriate probe. The cloning can be performed by PCR amplification using appropriate primers and subsequent ligation to an appropriate vector. The cloned nucleic acid may be further subcloned into another vector.

Commercially available plasmid vectors can be used, such as pBlue-Script™ SK(+) (Stratagene), pGEM-T (Promega), pAmp (TM: Gibco-BRL), p-Direct (Clontech), or pCR2.1-TOPO (Invitrogen). In PCR amplification, primers may be any regions of, e.g., the nucleotide sequence set forth in SEQ ID NO: 4. For example, NotI-PAH1-1-F: 5'-GCGGCCGCATGCAGTCCGTGGGAAG-3' (SEQ ID NO: 15) can be used as an upstream primer, and MaPAH1-1-10R: 5'-TTCTTGAGTAGCTGCTGTTGTTCG-3' (SEQ ID NO: 16) can be used as a downstream primer. Then, PCR is performed using cDNA prepared from *M. alpina* cells with the primers above, DNA polymerase, and any other substance. Although this procedure can be readily performed by those skilled in the art according to, e.g., "Molecular Cloning, A Laboratory Manual 3rd ed." (Cold Spring Harbor Press (2001)), PCR conditions in the present invention may be, for example, as follows:

Denaturation temperature: 90° C. to 95° C.,

Annealing temperature: 40° C. to 60° C.,

Elongation temperature: 60° C. to 75° C., and

Number of cycles: 10 or more cycles.

The resulting PCR product can be purified by a known method, for example, using a kit such as GENECLEAN kit

(Funakoshi Co., Ltd.), QIAquick PCR purification (QIAGEN), or ExoSAP-IT (GE Healthcare Bio-Sciences)); a DEAE-cellulose filter; or a dialysis tube. In the case of using an agarose gel, the PCR product is subjected to agarose gel electrophoresis, and nucleotide sequence fragments are cut out from the agarose gel and are purified, for example, with a GENECLEAN kit (Funakoshi Co., Ltd.) or a QIAquick Gel extraction kit (QIAGEN) or by a freeze-squeeze method.

The nucleotide sequence of the cloned nucleic acid can be determined with a nucleotide sequencer.

Vector Construction for Pap Expression and Preparation of Transformant

The present invention also provides a recombinant vector containing a nucleic acid encoding PAP of the present invention. The present invention further provides a transformant transformed with such a recombinant vector.

The recombinant vector and transformant can be prepared as follows: A plasmid having a nucleic acid encoding the PAP of the present invention is digested with a restriction enzyme. Examples of the restriction enzyme include, but not limited to, EcoRI, KpnI, BamHI, and SalI. The end may be blunted with T4 polymerase. A digested DNA fragment is purified by agarose gel electrophoresis. This DNA fragment is incorporated into an expression vector by a known method in order to prepare a vector for PAP expression. This expression vector is introduced into a host cell to prepare a transformant, which is provided for expression of a desired protein.

In this case, the expression vector and the host may be any types that allow expression of a desired protein. Examples of the host include fungi, bacteria, plants, animals, and cells thereof. Examples of fungi include filamentous fungi such as lipid-producing *M. alpina* and yeast strains such as *Saccharomyces cerevisiae*. Examples of bacteria include *Escherichia coli* and *Bacillus subtilis*. Further examples of plants include oil plants such as rapeseed, soybean, cotton, safflower, and flax.

As lipid-producing microorganisms, for example, strains described in MYCOTAXON, Vol. XLIV, NO. 2, pp. 257-265 (1992) can be used, and specific examples thereof include microorganisms belonging to the genus *Mortierella* such as microorganisms belonging to subgenus *Mortierella*, e.g., *Mortierella elongata* IFO8570, *Mortierella exigua* IFO8571, *Mortierella hygrophila* IFO5941, *Mortierella alpina* IFO8568, ATCC16266, ATCC32221, ATCC42430, CBS 219.35, CBS224.37, CBS250.53, CBS343.66, CBS527.72, CBS528.72, CBS529.72, CBS608.70, and CBS754.68; and microorganisms belonging to subgenus *Micromucor*, e.g., *Mortierella isabellina* CBS194.28, IFO6336, IFO7824, IFO7873, IFO7874, IFO8286, IFO8308, IFO7884, *Mortierella nana* IFO8190, *Mortierella ramanniana* IFO5426, IFO8186, CBS112.08, CBS212.72, IFO7825, IFO8184, IFO8185, IFO8287, and *Mortierella vinacea* CBS236.82. In particular, *Mortierella alpina* is preferred.

When a fungus is used as a host, the nucleic acid of the present invention is preferably self-replicable in the host or preferably has a structure insertable onto the fungal chromosome. Preferably, the nucleic acid also includes a promoter and a terminator. When *M. alpina* is used as a host, for example, pD4, pDuraSC, or pDura5 can be used as the expression vector. Any promoter that allows expression in the host can be used, and examples thereof include promoters derived from *M. alpina*, such as histonH4.1 gene promoter, GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene promoter, and TEF (translation elongation factor) gene promoter.

Examples of the method introducing a recombinant vector into filamentous fungi such as *M. alpina* include electroporation, a spheroplast method, a particle delivery method, and direct microinjection of DNA into nuclei. In the case of using an auxotrophic host strain, the transformed strain can be obtained by selecting a strain that grows on a selective medium lacking a certain nutrient(s). Alternatively, in transformation of using a drug resistant-marker gene, a colony of drug-resistant cells can be obtained by culturing the host cells in a selective medium containing the drug.

When yeast is used as a host, for example, pYE22m can be used as the expression vector. Alternatively, commercially available yeast expression vectors such as pYES (Invitrogen) or pESC(STRATAGENE) may be used. Examples of the host suitable for the present invention include, but not limited to, *Saccharomyces cerevisiae* strain EH13-15 (trp1, MATα). The promoter that can be used is, for example, a promoter derived from yeast, such as GAPDH promoter, gall promoter, or gal10 promoter.

Examples of the method introducing a recombinant vector into yeast include a lithium acetate method, electroporation, a spheroplast method, dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, encapsulation of polynucleotide(s) in liposomes, and direct microinjection of DNA into nuclei.

When a bacterium such as *E. coli* is used as a host, for example, pGEX or pUC18 available from Pharmacia can be used as the expression vector. The promoter that can be used include those derived from, for example, *E. coli* or phage, such as trp promoter, lac promoter, PL promoter, and PR promoter. Examples of the method of introducing a recombinant vector into bacteria include electroporation and calcium chloride methods.

Method of Preparing Fatty Acid Composition of the Present Invention

The present invention provides a method of preparing a fatty acid composition from the transformant described above, i.e., a method of preparing a fatty acid composition from a cultured product obtained by culturing the transformant. The fatty acid composition contains an assembly of one or more fatty acids therein. The fatty acids may be free fatty acids or may be present in the form of lipids containing fatty acids such as triglyceride or phospholipid. Specifically, the fatty acid composition of the present invention can be prepared by the following method. Alternatively, the fatty acid composition can also be prepared by any other known method.

The medium used for culturing an organism expressing PAP may be any culture solution (medium) that has an appropriate pH and osmotic pressure and contains biomaterials such as nutrients, trace elements, serum, and antibiotics necessary for growth of each host. For example, in the case of expressing PAP by transforming yeast, unlimited examples of the medium include SC-Trp medium, YPD medium, and YPD5 medium. The composition of a specific medium, for example, SC-Trp medium, is as follows: One liter of the medium includes 6.7 g of yeast nitrogen base w/o amino acids (DIFCO), 20 g of glucose, and 1.3 g of amino acid powder (a mixture of 1.25 g of adenine sulfate, 0.6 g of arginine, 3 g of aspartic acid, 3 g of glutamic acid, 0.6 g of histidine, 1.8 g of leucine, 0.9 g of lysine, 0.6 g of methionine, 1.5 g of phenylalanine, 11.25 g of serine, 0.9 g of tyrosine, 4.5 g of valine, 6 g of threonine, and 0.6 g of uracil).

Any culture conditions which are suitable for host growth and adequate for stably maintaining the generated enzyme may be employed. Specifically, individual conditions including anaerobic degree, culture period, temperature, humidity, and static culture or shake culture can be adjusted. Culture may be accomplished under the same conditions (one-step culture) or by so-called two-step or three-step culture using two or more different culture conditions. For large-scale culture, two- or more-step culture is preferred because of its high culture efficiency.

In two-step culture using yeast as the host, the fatty acid composition of the present invention can be prepared as follows: As pre-culture, a colony of a transformant is inoculated in, for example, the SC-Trp medium and shake-cultured at 30° C. for two days. Subsequently, 500 μL of the pre-culture solution as main culture is added to 10 mL of YPD5 (2% yeast extract, 1% polypeptone, and 5% glucose) medium, followed by shake culture at 30° C. for two days.

Fatty Acid Composition of the Present Invention

The present invention also provides a fatty acid composition as an assembly of one or more fatty acids in cells expressing PAP of the present invention, preferably, a fatty acid composition obtained by culturing a transformant expressing PAP of the present invention. The fatty acids may be free fatty acids or may be present in the form of lipids containing fatty acids such as triglyceride or phospholipid.

The fatty acids contained in the fatty acid composition of the present invention are linear or branched monocarboxylic acids of long-chain carbohydrates, and examples thereof include, but not limited to, myristic acid (tetradecanoic acid) (14:0), myristoleic acid (tetradecenoic acid) (14:1), palmitic acid (hexadecanoic acid) (16:0), palmitoleic acid (9-hexadecenoic acid) (16:1), stearic acid (octadecanoic acid) (18:0), oleic acid (cis-9-octadecenoic acid) (18:1(9)), vaccenic acid (11-octadecenoic acid) (18:1(11)), linolic acid (cis,cis-9,12 octadecadienoic acid) (18:2(9,12)), α-linolenic acid (9,12,15-octadecatrienoic acid) (18:3(9,12,15)), γ-linolenic acid (6,9,12-octadecatrienoic acid) (18:3(6,9,12)), stearidonic acid (6,9,12,15-octadecatetraenoic acid) (18:4(6,9,12,15)), arachidic acid (icosanoic acid) (20:0), (8,11-icosadienoic acid) (20:2(8,11)), mead acid (5,8,11-icosatrienoic acid) (20:3(5,8,11)), dihomo-γ-linolenic acid (8,11,14-icosatrienoic acid) (20:3(8,11,14)), arachidonic acid (5,8,11,14-icosatetraenoic acid) (20:4(5,8,11,14)), eicosatetraenoic acid (8,11,14,17-icosatetraenoic acid) (20:4(8,11,14,17)), eicosapentaenoic acid (5,8,11,14,17-icosapentaenoic acid) (20:5(5,8,11,14,17)), behenic acid (docosanoic acid) (22:0), (7,10,13,16-docosatetraenoic acid) (22:4(7,10,13,16)), (7,10,13,16,19-docosapentaenoic acid) (22:5(7,10,13,16,19)), (4,7,10,13,16-docosapentaenoic acid) (22:5(4,7,10,13,16)), (4,7,10,13,16,19-docosahexaenoic acid) (22:6(4,7,10,13,16,19)), lignoceric acid (tetracosanoic acid) (24:0), nervonic acid (cis-15-tetradocosanoic acid) (24:1), and cerotic acid (hexacosanoic acid) (26:0). Note that the substance names are common names defined by the IUPAC Biochemical Nomenclature, and their systematic names are given in parentheses along with numerics denoting the number of carbons and the positions of double bonds.

The fatty acid composition of the present invention may be composed of any number and any type of fatty acids, as long as it is a combination of one or more fatty acids selected from the fatty acids mentioned above.

Food or Other Products Comprising Fatty Acid Composition of the Present Invention

The present invention also provides a food product comprising the fatty acid composition described above. The fatty acid composition of the present invention can be used for production of food products containing fats and oils and production of industrial raw materials (for example, raw materials for cosmetics, pharmaceuticals (e.g., external applications for the skin), and soaps), in usual methods. Cosmetics (cosmetic compositions) or pharmaceuticals (pharmaceutical compositions) may be formulated into any dosage form including, but not limited to, solutions, pastes, gels, solids, and powders. Examples of the forms of food products include pharmaceutical formulations such as capsules; natural liquid diets, semi-digested nutritious diets, and elemental nutritious diets where the fatty acid composition of the present invention is blended with proteins, sugars, fats, trace elements, vitamins, emulsifiers, and flavorings; and processed forms such as drinkable preparations and enteral nutrients.

Moreover, examples of the food product of the present invention include, but not limited to, nutritional supplements, health food, functional food, children's food, modified milk for infants, modified milk for premature infant, and geriatric food. Throughout the specification, the term "food" is used as a collective term for edible materials in the form of a solid, a fluid, a liquid, or a mixture thereof.

The term "nutritional supplements" refers to food products enriched with specific nutritional ingredients. The term "health food" refers to food products that are healthful or good for health and encompasses nutritional supplements, natural food, and diet food. The term "functional food" refers to food products for supplying nutritional ingredients that assist body control functions and is synonymous with food for specified health use. The term "children's food" refers to food products given to children up to about 6 years old. The term "geriatric food" refers to food products treated to facilitate digestion and absorption thereof, compared to untreated food. The term "modified milk for infants" refers to modified milk given to children up to about one year old. The term "modified milk for premature infants" refers to modified milk given to premature infants until about 6 months after birth.

Examples of these food products include natural food (treated with fats and oils) such as meat, fish, and nuts; food supplemented with fats and oils during preparation, such as Chinese foods, Chinese noodles, and soups; food products prepared using fats and oils as heating media, such as tempura (deep-fried fish and vegetables), deep-fried food, fried tofu, Chinese fried rice, doughnuts, and Japanese fried dough cookies (karinto); fat- and oil-based food or processed food supplemented with fats and oils during processing, such as butter, margarine, mayonnaise, dressing, chocolate, instant noodles, caramel, biscuits, cookies, cake, and ice cream; and food sprayed or coated with fats and oils upon finishing, such as rice crackers, hard biscuits, and sweet bean paste bread. However, the food products of the present invention are not limited to food containing fats and oils, and other examples thereof include agricultural food products such as bakery products, noodles, cooked rice, sweets (e.g., candies, chewing gums, gummies, tablets, Japanese sweets), tofu, and processed products thereof; fermented food products such as refined sake, medicinal liquor, seasoning liquor (mirin), vinegar, soy sauce, and miso; livestock food products such as yogurt, ham, bacon, and sausage; seafood products such as fish paste (kamaboko), deep-fried fish paste (ageten), and fish cake (hanpen); and fruit drinks, soft drinks, sports drinks, alcoholic beverages, and tea.

Method for Strain Evaluation and Selection Using Pap-Encoding Nucleic Acid or PAP Protein of the Present Invention

The present invention also provides a method of evaluating or selecting a lipid-producing fungus using the PAP-encoding nucleic acid or PAP protein of the present invention. Details are given below.

(1) Method for Evaluation

One embodiment of the present invention is a method of evaluating a lipid-producing fungus using the PAP-encoding nucleic acid or PAP protein of the present invention. In the method for evaluation of the present invention, for example, a lipid-producing fungus strain as a test strain is evaluated for the activity of the present invention using primers or probes designed based on the nucleotide sequence of the present invention. Such evaluation can be performed by known procedures, for example, described in International Publication No. WO01/040514 and JP-A-8-205900. The method for evaluation will be briefly described below.

The first step is preparation of a genome of a test strain. The genome can be prepared by any known method such as a Hereford method or a potassium acetate method (see, e.g., Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, p. 130 (1990)).

Primers or probes are designed based on the nucleotide sequence of the present invention, preferably the sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6. These primers or probes may be any regions of the nucleotide sequence of the present invention and may be designed by a known procedure. The number of nucleotides in a polynucleotide used as a primer is generally 10 or more, preferably 15 to 25. The number of nucleotides appropriate for a region to be flanked by primers is generally 300 to 2000.

The primers or probes prepared above are used to examine whether the genome of a test strain contains a sequence specific to the nucleotide sequence of the present invention. The sequence specific to the nucleotide sequence of the present invention can be detected by a known procedure. For example, a polynucleotide containing a part or all of the sequence specific to the nucleotide sequence of the present invention or a polynucleotide containing a nucleotide sequence complementary to the nucleotide sequence is used as one primer, and a polynucleotide containing a part or all of a sequence located upstream or downstream of this sequence or a polynucleotide containing a nucleotide sequence complementary to the nucleotide sequence is used as the other primer, and a nucleic acid from the test strain is amplified by PCR or other techniques. Further, for example, the presence or absence of an amplification product and the molecular weight of an amplification product can be measured.

PCR conditions suitable for the method of the present invention are not particularly limited and may be, for example, as follows:

Denaturation temperature: 90° C. to 95° C.

Annealing temperature: 40° C. to 60° C.

Elongation temperature: 60° C. to 75° C.

Number of cycles: 10 or more cycles.

The resulting reaction products can be separated by electrophoresis on an agarose gel or any other process to determine the molecular weight of the amplification product. The test strain can be predicted or evaluated for the activity of the present invention by confirming whether the molecular weight of the amplification product is enough for covering a nucleic acid molecule corresponding to a region specific to the nucleotide sequence of the present invention. Furthermore, the activity of the present invention can be predicted or evaluated with higher accuracy by analyzing the nucleotide sequence of the amplification product by the method described above. The method of evaluating the activity of the present invention is as described above.

Alternatively, in the evaluation according to the present invention, a test strain can be evaluated for the activity of the present invention by culturing the test strain and measuring the expression level of PAP encoded by the nucleotide sequence of the present invention, e.g., the sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6. The expression level of PAP can be measured by culturing a test strain under appropriate conditions and quantifying mRNA or protein for PAP. The mRNA or protein can be quantified by a known procedure. For example, mRNA can be quantified by Northern hybridization or quantitative RT-PCR, and protein can be quantified by Western blotting (Current Protocols in Molecular Biology, John Wiley & Sons, 1994-2003).

(2) Method for Selection

Another embodiment of the present invention is a method of selecting a lipid-producing fungus using the PAP-encoding nucleic acid or PAP protein of the present invention. In the selection according to the present invention, a strain having a desired activity can be selected by culturing a test strain, measuring the expression level of PAP encoded by the nucleotide sequence of the present invention, e.g., sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 6, and selecting a strain of a desired expression level. Alternatively, a desired strain can be selected by establishing a standard strain, culturing the standard strain and a test strain separately, measuring the expression level of each strain, and comparing the expression level of the standard strain with that of the test strain. Specifically, for example, a standard strain and test strains are cultured under appropriate conditions, and the expression level of each strain is measured. A strain exhibiting a desired activity can be selected by selecting a test strain showing higher or lower expression than the standard strain does. The desired activity can be determined by, for example, measuring the expression level of PAP and the composition of fatty acids produced by PAP, as described above.

In the selection according to the present invention, a test strain having a desired activity can be selected by culturing test strains and selecting a strain having high or low activity of the present invention. A desired activity can be determined by, for example, measuring the expression level of PAP and the composition of fatty acids produced by PAP, as described above.

Examples of the test strain and the standard strain include, but not limited to, strains transformed with the vector of the present invention, strains modified to suppress expression of the nucleic acid of the present invention, mutagenized strains, and naturally mutated strains. The activity of the present invention can be measured by, for example, the method described in the "Nucleic acid encoding phosphatidic acid phosphatase of the present invention" in the specification. Examples of the mutagenesis include, but not limited to, physical methods such as irradiation with ultraviolet light or radiation; and chemical methods by treatment with a chemical such as EMS (ethylmethane sulfonate) or N-methyl-N-nitrosoguanidine (see, e.g., Yasuji Oshima ed., Biochemistry Experiments vol. 39, Experimental Protocols for Yeast Molecular Genetics, pp. 67-75, Japan Scientific Societies Press).

Examples of the strain used as the standard strain of the present invention or the test strain include, but not limited to, the lipid-producing fungus and yeast described above. Specifically, the standard strain and the test strain may be any

combination of strains belonging to different genera or species, and one or more test strains may be simultaneously used.

The present invention will now be described in more detail by the following examples, which are not intended to limit the scope of the invention.

EXAMPLES

Example 1

Genomic Analysis of *M. alpina*

M. alpina strain 1S-4 was inoculated into 100 mL of a GY2:1 medium (2% glucose, 1% yeast extract, pH 6.0) and was shake-cultured at 28° C. for 2 days. The cells were collected by filtration and genomic DNA was prepared by using DNeasy (QIAGEN).

The nucleotide sequence of the genome DNA was determined using a Roche 454 GS FLX Standard. On this occasion, the nucleotide of a fragment library was sequenced in two runs, and the nucleotide of a mate pair library was sequenced in three runs. The resulting nucleotide sequences were assembled to obtain 300 supercontigs.

Example 2

Synthesis of cDNA and Construction of cDNA Library

M. alpina strain 1S-4 was inoculated into 100 mL of a medium (1.8% glucose, 1% yeast extract, pH 6.0) and was shake-cultured at 28° C. for 4 days. The cells were collected by filtration, and total RNA was prepared by a guanidine hydrochloride/CsCl method.

From the total RNA, cDNA was synthesized by reverse transcription with SuperScript II RT (Invitrogen) using a random hexamer. In addition, from the total RNA, poly(A)$^+$ RNA was purified using an Oligotex-dT30<Super>mRNA Purification Kit (Takara Bio Inc.). A cDNA library was constructed using a ZAP-cDNA GigapackIII Gold Cloning Kit (STRATAGENE).

Example 3

Search for Homolog of *S. cerevisiae*-Derived PAH1

The amino acid sequence of a gene having the PAP activity of *Saccharomyces cerevisiae*, PAH1 (YMR165C) (may be also referred to as ScPAH1 in the specification), was subjected to tblastn analysis against *M. alpina* strain 1S-4 genome nucleotide sequences. As a result, supercontigs including the sequences set forth in SEQ ID NO: 5 and SEQ ID NO: 10 gave a hit. The gene relating to SEQ ID NO: 5 was named MaPAH1.1, and the gene relating to SEQ ID NO: 10 was named MaPAH1.2.

Example 4

Cloning of MaPAH1.1 and MaPAH1.2

(1) Preparation of Probe

In order to clone cDNAs of the MaPAH1.1 gene and the MaPAH1.2 gene, nucleotide sequences set forth in SEQ ID NO: 5 and SEQ ID NO: 10 and the following primers determined based on the results of the BLAST analysis above were prepared.

(SEQ ID NO: 11)
MaPAH1-1-3F: 5'-CGCCAATACATTGACGTTTTCAG-3'

(SEQ ID NO: 12)
MaPAH1-1-5R: 5'-AGTTCCAGTCATTGAACTCGGGTGC-3'

(SEQ ID NO: 13)
MaPAH1-2-3F: 5'-GAGCCCAGTTGACCTTTGAGGCATTC-3'

(SEQ ID NO: 14)
MaPAH1-2-5R: 5'-CACTGAGAACGAGACCGTGTTGGCG-3'

PCR was performed with ExTaq (Takara Bio Inc.) using the cDNA library constructed in Example 2 as a template and a combination of primer MaPAH1-1-3F and primer MaPAH1-1-5R or a combination of primer MaPAH1-2-3F and primer MaPAH1-2-5R at 94° C. for 2 min and then 30 cycles of (94° C. for 30 sec, 55° C. for 30 sec, and 72° C. for 2 min). The DNA fragment of about 0.6 kbp obtained in each combination was cloned with a TOPO-TA cloning Kit (Invitrogen), and the nucleotide sequence of the insert of the resulting plasmid was determined. The plasmid, obtained by the former combination of the primer, having a sequence corresponding to the nucleotides 2352 to 3010 of SEQ ID NO: 4 was identified as pCR-MaPAH1.1-P; and the plasmid, obtained by the latter combination of the primers, having a sequence corresponding to the nucleotides 1615 to 2201 of SEQ ID NO: 9 was identified as pCR-MaPAH1.2-P.

Subsequently, probes were produced by PCR using these plasmids as templates and the primers in the above. In the reaction, ExTaq (Takara Bio Inc., Japan) was used, except that a PCR labeling mix (Roche Diagnostics) was used instead of the attached dNTP mix for labeling DNAs to be amplified with digoxigenin (DIG) to prepare an MaPAH1.1 probe and an MaPAH1.2 probe. The cDNA library was screened with these probes.

Hybridization conditions were set as follows:

Buffer: 5×SSC, 1% SDS, 50 mM Tris-HCl (pH 7.5), 50% formamide,

Temperature: 42° C. (overnight), and

Washing conditions: in 0.2×SSC, 0.1% SDS solution (65° C.) for 20 min (three times).

A DIG nucleic acid detection kit (Roche Diagnostics) was used for detection. Plasmids were cut out by in vivo excision from phage clones obtained by screening to obtain each plasmid DNA. A plasmid having the longest insert among the plasmids obtained by screening with the MaPAH1.1 probe had a sequence of the positions 1307th and after in the sequence set forth in SEQ ID NO: 4 and was named plasmid pB-MaPAH1.1p. The results of comparison with the amino acid sequence of ScPAH1 suggest that this plasmid pB-MaPAH1.1p does not contain a region encoding the N-terminal of PAH1.1. Comparison of the genomic sequence (SEQ ID NO: 5), which was expected to have the MaPAH1.1 gene from the results of BLAST analysis, with the N-terminal sequence of the amino acid sequence of ScPAH1 suggest that ATG at the 1 to 3 positions in the sequence set forth in SEQ ID NO: 5 is the start codon. Each frame of the plasmid pB-MaPAH1.1p was translated into an amino acid sequence. The amino acid sequence was compared with the amino acid sequence of ScPAH1 protein derived from *S. cerevisiae*. The results suggest that the TGA at the 3985 to 3987 positions in the sequence set forth in SEQ ID NO: 4 is the stop codon. Accordingly, in order to clone the full-length cDNA, the following primers were designed:

(SEQ ID NO: 15)
NotI-PAH1-1-F: 5'-GCGGCCGCATGCAGTCCGTGGGAAG-3',
and

(SEQ ID NO: 16)
MaPAH1-1-10R: 5'-TTCTTGAGTAGCTGCTGTTGTTCG-3'.

PCR was performed with ExTaq (Takara Bio Inc.) using the cDNA above as a template and a combination of primer NotI-PAH1-1-F and primer MaPAH1-1-10R at 94° C. for 2 min and then 30 cycles of (94° C. for 30 sec, 55° C. for 30 sec, and 72° C. for 2 min). The resulting DNA fragment of about 1.5 kbp was cloned with a TOPO-TA cloning Kit (Invitrogen), and the nucleotide sequence of the inserted part was determined. The plasmid that cloned the DNA fragment including the sequence of nucleotides 1 to 1500 of SEQ ID NO: 4 was identified as pCR-MaPAH1.1-Np. Subsequently, a DNA fragment of about 1.4 kbp obtained by digestion of plasmid pCR-MaPAH1.1-Np with restriction enzymes NotI and XhoI, a DNA fragment of about 3.7 kbp obtained by digestion of plasmid pB-MaPAH1.1p with restriction enzymes NotI and BamHI, and a DNA fragment of about 2.1 kb obtained by digestion of plasmid pB-MaPAH1.1p with restriction enzymes XhoI and BamHI were linked using ligation high (TOYOBO) to prepare plasmid pB-MaPAH1.1 cDNA, which probably contain the full-length cDNA of MaPAH1.1. A cDNA sequence including the full-length ORF of MaPAH1.1 is shown in SEQ ID NO: 4.

Separately, a plasmid having the longest insert among the plasmids obtained by screening with the MaPAH1.2 probe had the nucleotide sequence set forth in SEQ ID NO: 9. The results of comparison of this plasmid with the sequence of ScPAH1 derived from *S. cerevisiae* suggest that the plasmid has cDNA including the full-length ORF of MaPAH1.2. This plasmid was identified as pB-MaPAH1.2 cDNA.

(2) Sequence Analysis

The cDNA sequence (SEQ ID NO: 4) of the MaPAH1.1 gene includes CDS (SEQ ID NO: 3) consisting of a sequence of the nucleotides 1 to 3987 and ORF (SEQ ID NO: 1) consisting of a sequence of the nucleotides 1 to 3984. A deduced amino acid sequence encoded by the MaPAH1.1 gene is shown in SEQ ID NO: 2. The genomic sequence of the MaPAH1.1 gene was compared with the ORF sequence (FIG. **1**). The results suggest that the genomic sequence of the MaPAH1.1 gene is composed of eleven exons and ten introns.

The cDNA sequence (SEQ ID NO: 9) of the MaPAH1.2 gene includes CDS (SEQ ID NO: 8) consisting of a sequence of the nucleotides 72 to 3791 and ORF (SEQ ID NO: 6) consisting of a sequence of the nucleotides 72 to 3788. A deduced amino acid sequence encoded by the MaPAH1.2 gene is shown in SEQ ID NO: 7. The genomic sequence of the MaPAH1.2 gene was compared with the ORF sequence (FIG. **2**). The genomic sequence of the MaPAH1.2 gene is composed of eight exons and seven introns.

The cDNA sequences of MaPAH1.1 and MaPAH1.2 and deduced amino acid sequences thereof are respectively shown in FIG. **3** and FIG. **4**.

The deduced amino acid sequences of MaPAH1.1 and MaPAH1.2 were subjected to homology search against amino acid sequences in GenBank with the BLASTp program. Both amino acid sequences gave a hit with nuclear elongation and deformation protein 1 putative protein (AAW42851) derived from *Cryptococcus neoformans* var. *neoformans* JEC21 with the highest scores, but the identities thereof were low, i.e., 25.9% and 26.6%, respectively.

The amino acid sequences of MaPAH1.1 and MaPAH1.2 derived from *M. alpina* of the present invention have identities of 22.7% and 22.5%, respectively, with the amino acid sequence of ScPAH1 protein, which has been functionally analyzed, among PAP1 homologs of fungi. The amino acid sequences of MaPAH1.1 and MaPAH1.2 derived from *M. alpina* in the present invention were compared with the amino acid sequences of known ScPAH1 and mouse-derived lipin (FIG. **5**). In the PAP1 family enzymes, the amino acid sequence of the N-terminal region is well conserved and is called lipin, N-terminal conserved region (pfam04571). In also MaPAH1.1 and MaPAH1.2 derived from *M. alpina* of the present invention, the known enzyme and the N-terminal region are relatively well conserved. In addition, the DIDGT sequence indicated with double underline in FIG. **5** is haloacid dehalogenase (HAD)-like protein superfamily enzyme and is consistent with the motif of the conserved DXDX(T/V) catalytic site.

The CDS sequences of MaPAH1.1 and MaPAH1.2 were compared with each other to show an identity of 54.7% (FIG. **6**), while the identity between the deduced amino acid sequences was 35.6% (FIG. **7**).

Example 5

Expression of MaPAH1.1 and MaPAH1.2 in Yeast

Construction of Expression Vector of MaPAH1.1 and MaPAH1.2:

In order to express MaPAH1.1 in yeast, expression vectors were constructed as follows.

Yeast expression vector pYE22m (Biosci. Biotech. Biochem., 59, 1221-1228, 1995) was digested with a restriction enzyme EcoRI, and the ends were blunted with a Blunting Kit (TaKaRa Bio Inc.). The resulting fragment and a linker, pNotI, phosphorylated (8-mer) (TaKaRa Bio Inc.) were linked to each other using ligation high (TOYOBO) to construct vector pYE22mN. The vector pYE22mN was digested with restriction enzymes NotI and KpnI, and the resulting fragment was linked to a DNA fragment of about 4.2 kbp obtained by digestion of plasmid pB-MaPAH1.1 cDNA with restriction enzymes NotI and KpnI to provide plasmid pYE-MaPAH1.1. Separately, vector pYE22mN was digested with restriction enzymes NotI and KpnI, and the resulting fragment was linked to a DNA fragment of about 3.8 kbp obtained by digestion of plasmid pB-MaPAH1.2 cDNA with restriction enzymes NotI and KpnI to provide plasmid pYE-MaPAH1.2.

Preparation of *S. cerevisiae* ΔScpah1:URA3 Strain

In order to clone an ScPAH1 gene derived from *S. cerevisiae* strain S288C, the following primers were prepared:

Primer KpnI-PAH1-F:
(SEQ ID NO: 17)
5'-GGTACCATGCAGTACGTAGGCAGAGCTC-3',
and

Primer XhoI-PAH1-R:
(SEQ ID NO: 18)
5'-CTCGAGTTAATCTTCGAATTCATCTTCG-3'.

S. cerevisiae strain S288C was cultured in an YPD (2% yeast extract, 1% polypeptone, 2% glucose) liquid medium at 30° C. overnight. From the cells, DNA was extracted using Dr. GenTLE (from yeast) (TaKaRa Bio Inc.), and the ScPAH1 gene was amplified by PCR with ExTaq using the resulting DNA as a template and primers KpnI-PAH1-F and

XhoI-PAH1-R. The resulting DNA fragment of about 2.5 kbp was cloned using a TOPO TA cloning Kit, and a clone having a correct nucleotide sequence was identified as pCR-ScPAH1. A DNA fragment of about 0.4 kbp obtained by digestion of pCR-ScPAH1 with restriction enzymes EcoRI and EcoRV and a DNA fragment of about 2.1 kbp obtained by digestion of pCR-ScPAH1 with restriction enzymes EcoRV and XhoI were ligated to vector pBluescriptIISK+digested by restriction enzymes EcoRI and XhoI to prepare plasmid pBScPAH1. Plasmid pBScPAH1 was digested with restriction enzymes EcoRV and HincII and was ligated to a DNA fragment of about 1.2 kbp obtained by digestion of plasmid pURA34 (Japanese Unexamined Patent Application Publication No. 2001-120276) with a restriction enzyme HindIII and then blunt-ended. The resulting product having the URA3 gene in the same direction as that of the ScPAH1 gene was determined as plasmid pBΔpah1:URA3. Subsequently, *S. cerevisiae* strain YPH499 (ura3-52 lys2-801amber ade2-101ochre trp1-Δ63 his3-Δ200 leu2-Δ1 a) (STARATAGENE), as a host, was transformed with a DNA fragment obtained by digestion of plasmid pBΔpah1:URA3 with a restriction enzyme EcoRI. Transformed strain was selected by the ability to grow on an SC-Ura agar medium (one liter of the medium includes 6.7 g of yeast nitrogen base w/o amino acids (DIFCO), 20 g of glucose, 1.3 g of amino acid powder (a mixture of 1.25 g of adenine sulfate, 0.6 g of arginine, 3 g of aspartic acid, 3 g of glutamic acid, 0.6 g of histidine, 1.8 g of leucine, 0.9 g of lysine, 0.6 g of methionine, 1.5 g of phenylalanine, 11.25 g of serine, 0.9 g of tyrosine, 4.5 g of valine, 6 g of threonine, and 1.2 g of tryptophan), and an agar medium (2% agar)). A strain that was confirmed by PCR that the Δpah1:URA3 construction was introduced thereinto and that the ScPAH1 gene was disrupted was determined as a ΔScpah1:URA3 strain.

Acquisition of Transformed Strain:

The ΔScpah1:URA3 strain was used as a host and transformed with plasmid pYE22m, pYE-MaPAH1.1, or pYE-MaPAH1.2. Transformed strains were selected by the ability to grow on an SC-Ura, Trp agar medium (one liter of the medium includes 6.7 g of yeast nitrogen base w/o amino acids (DIFCO), 20 g of glucose, 1.3 g of amino acid powder (a mixture of 1.25 g of adenine sulfate, 0.6 g of arginine, 3 g of aspartic acid, 3 g of glutamic acid, 0.6 g of histidine, 1.8 g of leucine, 0.9 g of lysine, 0.6 g of methionine, 1.5 g of phenylalanine, 11.25 g of serine, 0.9 g of tyrosine, 4.5 g of valine, and 6 g of threonine), and an agar medium (2% agar)). Arbitrary two strains from the respective strains transformed with each plasmid (control strains transformed with plasmid pYE22m: C1 and C2, strains transformed with plasmid pYE-MaPAH1.1: MaPAH1.1-1 and MaPAH1.1-2, and strains transformed with plasmid pYE-MaPAH1.2: MaPAH1.2-1 and MaPAH1.2-2) were used the subsequent experiments.

Example 6

Measurement of Mg^{2+}-Dependent Phosphatidic Acid Phosphatase Activity (PAP1 Activity)

Each transformed yeast strain was inoculated into 100 mL of an SC-Ura, Trp liquid medium and shake-cultured at 30° C. for one day. A crude enzyme solution was prepared from the resulting culture solution as follows. In particular, the procedure was conducted at 4° C. or in ice. The cells were collected from the culture solution by centrifugation and were washed with water. Subsequently, the cells were suspended in 5 mL of buffer A (50 mM Tris-HCl (pH 7.5), 0.3 M sucrose, 10 mM mercaptoethanol, 0.5 mM phenylmethylsulfonyl fluoride (PMSF)). The cells were disrupted by treatment with a french press (Thermo Fisher Scientific), Mini-Cell, at 16 kPa three times. The cell lysate was subjected to centrifugation at 1500×g for 10 min, and the supernatant was collected as a crude enzyme solution. The concentration of protein contained in the crude enzyme solution was measured with Protein Assay CBB Solution (5×) (Nacalai Tesque).

The PAP1 activity was measured by a modified method by Gil-Soo, et al. (J. Biol. Chem., 282 (51), 37026-37035, (2007)) as follows. Since *S. cerevisiae* cannot synthesize linoleic acid, 1,2-dilinoleoyl-sn-glycero-3-phosphate (18:2-PA) was used as the substrate of PAP. Five hundred microliters of a reaction solution was used. The composition of the reaction solution was 100 μL of the crude enzyme solution, 50 mM Tris-HCl (pH 7.5), 100 μg/mL of 1,2-dilinoleoyl-sn-glycero-3-phosphate, monosodium salt (Avanti Polar Lipids, Inc.), 1 mM $MgCl_2$, and 10 mM 2-mercaptoethanol. The reaction solution was maintained at 25° C. for 30 min, and then the reaction was terminated by addition of chloroform:methanol (1:2). Lipids were extracted by a Bligh-Dyer method. The lipids were fractionated on a silica gel 60 plate (Merck) by thin layer chromatography (TLC) using hexane:diethyl ether:acetic acid=70:30:1 as the eluent. The lipids were visualized by spraying a primulin solution (0.015% primulin in aqueous 80% acetone) and then irradiated with UV light. The diacylglycerol (DG) fraction was scraped from the plate and fatty acids were converted to methyl ester by a hydrochloric acid/methanol method. Subsequently, fatty acid methyl ester was extracted with hexane, and hexane was distilled off, followed by gas chromatographic analysis.

Table 2 shows the amounts of linoleic acid transferred into the DG fraction per protein in the crude enzyme solution.

TABLE 2

Transformed strain	18:2 (μg/mg protein)
C1	15.43
C2	17.53
MaPAH1.1-1	56.03
MaPAH1.1-2	44.34
MaPAH1.2-1	19.45
MaPAH1.2-2	20.90

As shown in Table 2, in comparison with C1 and C2 transformed with pYE22m, the activity of converting 18:2-PA to dilinolein (18:2-DG) was about 3-fold in MaPAH1.1-1 and MaPAH1.1-2 expressing MaPAH1.1 and about 1.2-fold in MaPAH1.2-1 and MaPAH1.2-2 expressing MaPAH1.2. This suggests that MaPAH1.1 and MaPAH1.2 have PAP activity.

The dependency of the PAP activity on Mg^{2+} was investigated as follows: Five hundred microliters of a reaction solution was used. The reaction and analysis were performed under the same conditions as above except that the composition of the reaction solution was 100 μL of the crude enzyme solution, 50 mM Tris-HCl (pH 7.5), 100 μg/mL of 1,2-dilinoleoyl-sn-glycero-3-phosphate, monosodium salt (Avanti Polar Lipids, Inc.), 2 mM EDTA, and 10 mM 2-mercaptoethanol. Table 3 shows the amounts of linoleic acid transferred into the DG fraction per protein in the crude enzyme solution.

TABLE 3

Transformed strain	18:2 (μg/mg protein)
C1	11.17
C2	10.77
MaPAH1.1-1	13.06
MaPAH1.1-2	11.39
MaPAH1.2-1	12.52
MaPAH1.2-2	10.93

As shown in Table 3, in every strain, the activity of converting 18:2-PA to dilinolein (18:2-DG) was approximately the same.

This suggests that the PAP activity of MaPAH1.1 and MaPAH1.2 depends on Mg^{2+} and that MaPAH1.1 and MaPAH1.2 have PAP1 activity.

Example 7

Amount of Produced Triacylglycerol

Triacylglycerol (throughout the specification, referred to as triglyceride or TG), which is a reserve lipid, is a lipid obtained by further acylating diacylglycerol which is a product of PAP protein. The amounts of TG produced by yeast transformants in which MaPAH1.1 or MaPAH1.2 was highly expressed were measured.

The transformant cells, ScPAH1-deficient yeast strain host, were inoculated in 10 mL of an SD-Ura, Trp liquid medium and were statically cultured at 30° C. for 3 days. One milliliter of the culture solution was inoculated in 10 mL of a YPDA (2% yeast extract, 1% polypeptone, 2% glucose, 0.008% adenine sulfate) liquid medium, followed by shake culture at 30° C. for one day (n=3). The cells were collected by centrifugation of the culture solution, washed with water, and lyophilized. Chloroform and methanol (2:1) were added to the dried cells. The cells were repeatedly disrupted with glass beads, and lipids were extracted with 8 mL in total of a solvent. The extracted lipids were fractionated by TLC as in above, and the TG fraction was scraped and analyzed. Table 4 shows the results.

TABLE 4

Amount* of TG produced in each medium	
Transformed strain	mg/L
C1	11.01 ± 1.27
C2	11.54 ± 0.54
MaPAH1.1-1	16.01 ± 2.45
MaPAH1.1-2	17.09 ± 1.41
MaPAH1.2-1	14.29 ± 0.87
MaPAH1.2-2	13.32 ± 0.78

*In terms of fatty acid

As shown in Table 4, the amount of TG was about 1.5-fold in the MaPAH1.1 high expression strain and was about 1.2-fold in the MaPAH1.2 high expression strain compared with that in the control.

Example 8

Substrate Specificity of MaPAH1.1 and MaPAH1.2

The ΔScpah1:URA3 strain, the host, was transformed with plasmid pYE22m, pYE-MaPAH1.1, or pYE-MaPAH1.2. Four strains of each transformant were used in the following experiments. The strains transformed with plasmid pYE22m were used as a control.

The yeast transformants were each inoculated in 10 mL of an SC-Ura, Trp liquid medium and were statically cultured at 27.5° C. overnight. The resulting culture solutions were each inoculated in 40 mL of an SC-Ura, Trp liquid medium at an amount of 1/10 in duplicate and were statically cultured at 27.5° C. for two days. Crude enzyme solutions were prepared from the resulting culture solutions as in Example 6, and the protein concentrations thereof were measured.

The PAP1 activity was measured as in Example 6 except that 1,2-dilinoleoyl-sn-glycero-3-phosphate (18:2-PA) and 1,2-dioleoyl-sn-glycero-3-phosphate (18:1-PA) were used as substrates of PAP.

Tables 5 and Table 6 respectively show the amounts of linoleic acid (18:2) and oleic acid (18:1) transferred into the diacylglycerol (DG) fraction per crude enzyme solution protein.

TABLE 5

18:2 in DG per protein (μg/mg · protein)						
	Control		MaPAH1.1		MaPAH1.2	
Sample name	mean	SD	mean	SD	mean	SD
	13.72	2.74	25.50	6.75	18.19	1.43

TABLE 6

18:1 in DG per protein (μg/mg · protein)						
	Control		MaPAH1.1		MaPAH1.2	
Sample name	mean	SD	mean	SD	mean	SD
	15.14	0.88	29.16	7.04	16.69	1.05

When the substrate used was 18:2-PA, the activities of MaPAH1.1 and MaPAH1.2 derived from *Mortierella* were 1.9-fold and 1.3-fold, respectively, compared with that of the control.

When the substrate used was 18:1-PA, the activities of MaPAH1.1 and MaPAH1.2 were 1.9-fold and 1.1-fold, respectively, compared with that of the control. The 18:1 is a fatty acid that yeast intrinsically possesses and is therefore originally present in DG of the crude enzyme solution. However, no difference was observed in the amount of 18:1 in DG in the crude enzyme solution when the substrate was not added. Accordingly, it can be assumed that the differences in activity of MaPAH1.1 and MaPAH1.2 from the control shown in Table 6 are based on the effect against 18:1-PA added as a substrate.

In comparison of activities of the same enzyme against different substrates, MaPAH1.1 increased both 18:1 and 18:2 by 1.9-fold compared with the control, while MaPAH1.2 increased the amount of 18:1 by 1.1-fold and the amount of 18:2 by 1.3-fold compared with the control. This suggests that MaPAH1.1 exhibits its activity on both 18:1-PA and 18:2-PA equally, but in MaPAH1.2, the activity on 18:2-PA is higher than that on 18:1-PA.

These results suggest that MaPAH1.1 and MaPAH1.2 have PAP activity. In addition, MaPAH1.2 shows higher activity on 18:2-PA than on 18:1-PA, which suggests that MaPAH1.2 shows a higher activity on phosphatidic acid having a fatty acid portion with a higher degree of unsaturation.

SEQUENCE LISTING FREE TEXT

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SEQ ID NO: 12: primer
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SEQ ID NO: 15: primer
SEQ ID NO: 16: primer
SEQ ID NO: 17: primer
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SEQUENCE LISTING

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Glu Leu Asn Pro Ala Thr Leu Ser Gly Ala Ile Asp Val Val Val Val
            20                  25                  30

gag caa gcc gat ggt gaa tta gca tgc tca cca ttt cat gtc cgc ttt      144
Glu Gln Ala Asp Gly Glu Leu Ala Cys Ser Pro Phe His Val Arg Phe
        35                  40                  45

ggc aaa ctg agc att ctc cga ccg cag gaa aaa gtg gtg gag gtg acc      192
Gly Lys Leu Ser Ile Leu Arg Pro Gln Glu Lys Val Val Glu Val Thr
    50                  55                  60

gtc aac ggt cgc gtc gtt gat ttt cct atg aag gtt ggc gat gca ggc      240
Val Asn Gly Arg Val Val Asp Phe Pro Met Lys Val Gly Asp Ala Gly
65                  70                  75                  80

gaa gcc ttc ttt gtt ttt gag act gag cag gac gtg ccc gaa gag ttt      288
Glu Ala Phe Phe Val Phe Glu Thr Glu Gln Asp Val Pro Glu Glu Phe
                85                  90                  95

gcc acg tct cca cta gcg gga ccc aac aca gac aaa gtt gag gag gac      336
Ala Thr Ser Pro Leu Ala Gly Pro Asn Thr Asp Lys Val Glu Glu Asp
            100                 105                 110

att gac tat ctg gat cta gcc gaa ggg cat agc acc gtg aca tat ccg      384
Ile Asp Tyr Leu Asp Leu Ala Glu Gly His Ser Thr Val Thr Tyr Pro
        115                 120                 125

cct gac gat ata gtc tta gat gcg ggc tat gtc agc gcc cac agt ggg      432
Pro Asp Asp Ile Val Leu Asp Ala Gly Tyr Val Ser Ala His Ser Gly
    130                 135                 140

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His Gly Ser Glu Phe Glu Glu Asp Glu Arg Ala Asp Leu Ser Pro Glu
145                 150                 155                 160

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                165                 170                 175

aat gga caa ggg aga cac cta ggc agt gct aat gag gca aca acg tct      576
Asn Gly Gln Gly Arg His Leu Gly Ser Ala Asn Glu Ala Thr Thr Ser
            180                 185                 190

gta cat gct ttc atg gag cgg caa gtt caa cga tgg tcg ctt acc atg      624
Val His Ala Phe Met Glu Arg Gln Val Gln Arg Trp Ser Leu Thr Met
        195                 200                 205

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    210                 215                 220

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225                 230                 235                 240

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Ser Ser Gly Ser Leu Phe His Pro Lys Glu Gly Met Ile Met Asp Met
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Thr Gly Tyr Lys Thr Glu Asp Ser Asp Leu Asn Ser Asp Ala Ser Asp
        275                 280                 285

gaa cat gat gta ggc atg gct ggc gct ttg aat ggt cgc cat cgg cgc      912
Glu His Asp Val Gly Met Ala Gly Ala Leu Asn Gly Arg His Arg Arg
    290                 295                 300

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Lys Arg Ala Ala Arg Arg Lys Arg Arg Gly Pro Val His Gly Val Asn
305                 310                 315                 320

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Ser Gln Asp Asn Leu Ala Thr Glu Thr Pro Ser Ile Thr Ala His Val
                325                 330                 335

ctc agc agt ctc gac cct cgc ttg ccg ttg cga cct act gcg cga cct     1056
Leu Ser Ser Leu Asp Pro Arg Leu Pro Leu Arg Pro Thr Ala Arg Pro
            340                 345                 350

gct cta cgc ccc aaa gct aac aac ggg ttg ggc act cta ccg aat cgc     1104
Ala Leu Arg Pro Lys Ala Asn Asn Gly Leu Gly Thr Leu Pro Asn Arg
        355                 360                 365

cgt tcg tca tcg atg ccg aat ctt aaa gat ttc gta ggt gag aat aac     1152
Arg Ser Ser Ser Met Pro Asn Leu Lys Asp Phe Val Gly Glu Asn Asn
    370                 375                 380

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Ser Leu Ser Pro Ser Val Pro Ala Ile Met Arg Arg Phe Pro Ser Lys
385                 390                 395                 400

acg tta aac tca aag ttt tcc gca aga agc gac atc aaa gat ggg acc     1248
Thr Leu Asn Ser Lys Phe Ser Ala Arg Ser Asp Ile Lys Asp Gly Thr
                405                 410                 415

agt tca agc agc tcc gta gcc tcc tcg cct cca ccg tca gtt gcc aac     1296
Ser Ser Ser Ser Ser Val Ala Ser Ser Pro Pro Pro Ser Val Ala Asn
            420                 425                 430

cag cag agc cct aaa aac cgc cac cat cac cat cat cac cac aaa gag     1344
Gln Gln Ser Pro Lys Asn Arg His His His His His His His Lys Glu
        435                 440                 445

cac acc gaa gga agc cat ccc cgt cgc cac tcg cac aaa cct tca cag     1392
His Thr Glu Gly Ser His Pro Arg Arg His Ser His Lys Pro Ser Gln
    450                 455                 460

caa gtg caa gtg aaa aaa ccc ccg ccc aga tcc aat cca gct gtt aat     1440
Gln Val Gln Val Lys Lys Pro Pro Pro Arg Ser Asn Pro Ala Val Asn
465                 470                 475                 480

gcg ctg agc gat acg gag ctc gag tat caa acg ccg cga aca aca gca     1488
Ala Leu Ser Asp Thr Glu Leu Glu Tyr Gln Thr Pro Arg Thr Thr Ala
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gct act caa gaa tca gag tgg tcc tgg gga tgg ggc agc tta ccg gtt     1536
Ala Thr Gln Glu Ser Glu Trp Ser Trp Gly Trp Gly Ser Leu Pro Val
            500                 505                 510

aaa aat gac ggt cta ggc aca ggg gaa gca gat cac aag gag cat cac     1584
Lys Asn Asp Gly Leu Gly Thr Gly Glu Ala Asp His Lys Glu His His
        515                 520                 525

tct agt cat cca tca atc gac att cca gcc cca cgg aaa cct gtg ttg     1632
Ser Ser His Pro Ser Ile Asp Ile Pro Ala Pro Arg Lys Pro Val Leu
    530                 535                 540

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tgt ccg ggt gat gaa ttc gga aaa gat ttg gaa gcc agc gaa gca ttg     1728
Cys Pro Gly Asp Glu Phe Gly Lys Asp Leu Glu Ala Ser Glu Ala Leu
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ttt gcc acc aat cag gtt tcg ttc gat gag ttc gcg aaa gac cca ctc     1776
Phe Ala Thr Asn Gln Val Ser Phe Asp Glu Phe Ala Lys Asp Pro Leu
            580                 585                 590

aag act ctc aat aac aag aat ttg gtc tgc ctg atc aat gac cgg tat     1824
Lys Thr Leu Asn Asn Lys Asn Leu Val Cys Leu Ile Asn Asp Arg Tyr
        595                 600                 605

ttt act tgg aca gct gcg gga cca tat ctt tcc tca ctg atg ctc ttc     1872
Phe Thr Trp Thr Ala Ala Gly Pro Tyr Leu Ser Ser Leu Met Leu Phe
    610                 615                 620

cgg aag cct ctc tct gac gaa acg ctc cat cag ctt tca gcc aag gac     1920
Arg Lys Pro Leu Ser Asp Glu Thr Leu His Gln Leu Ser Ala Lys Asp
625                 630                 635                 640

tcg cgg cat cta tca gat cga ctc gct gtg caa gat gag ccc cca acc     1968
Ser Arg His Leu Ser Asp Arg Leu Ala Val Gln Asp Glu Pro Pro Thr
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cgt ttc ggc gct ctc tcc aga tgg cta agg gga tca caa acc tcg tcc     2016
Arg Phe Gly Ala Leu Ser Arg Trp Leu Arg Gly Ser Gln Thr Ser Ser
            660                 665                 670

caa ttg agc gcg atg gag caa ggg caa aga caa cgt act ccc agt acc     2064
Gln Leu Ser Ala Met Glu Gln Gly Gln Arg Gln Arg Thr Pro Ser Thr
        675                 680                 685

aac gat gcc ttg cag cct gct cag tta gag gag agt caa gct tta cag     2112
Asn Asp Ala Leu Gln Pro Ala Gln Leu Glu Glu Ser Gln Ala Leu Gln
    690                 695                 700

agc gtg aaa gtc gaa tcg att aag cac act tcc gga tca cat tca tca     2160
Ser Val Lys Val Glu Ser Ile Lys His Thr Ser Gly Ser His Ser Ser
705                 710                 715                 720

ctt cta cgc gct cct aaa cca atg act cgt agc acc tct ctg ccg atc     2208
Leu Leu Arg Ala Pro Lys Pro Met Thr Arg Ser Thr Ser Leu Pro Ile
                725                 730                 735

gac gaa ggg atc gcc ggg tct ata tca gac gag tac gct gga agc tcg     2256
Asp Glu Gly Ile Ala Gly Ser Ile Ser Asp Glu Tyr Ala Gly Ser Ser
            740                 745                 750

cct ccg aca cat tct gcg ctc aag agc agt aga cgg tat gcg aaa acg     2304
Pro Pro Thr His Ser Ala Leu Lys Ser Ser Arg Arg Tyr Ala Lys Thr
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ctt cgc ttg aca tct gaa cag ttg aaa tca cta aat ttg aaa aaa ggc     2352
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Ala Asn Thr Leu Thr Phe Ser Val Thr Ser Ser Tyr Gln Gly Lys Ala
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gtt tgt tcc gcc aaa ttg ttt ctg tgg gac cat gac tac caa gtc gtc     2448
Val Cys Ser Ala Lys Leu Phe Leu Trp Asp His Asp Tyr Gln Val Val
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ata tcg gac att gat ggc acg att aca aag tcg gac gct ctc gga cac     2496
Ile Ser Asp Ile Asp Gly Thr Ile Thr Lys Ser Asp Ala Leu Gly His
            820                 825                 830

atc ttt acc atg gca gga aag gat tgg acc cat tcg ggt gtc gcc aaa     2544
Ile Phe Thr Met Ala Gly Lys Asp Trp Thr His Ser Gly Val Ala Lys
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ctt tac acg gac atc gtc aac aat ggg tat cat att ttg tac ttg acc     2592
Leu Tyr Thr Asp Ile Val Asn Asn Gly Tyr His Ile Leu Tyr Leu Thr
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gtg gag caa aat aac tac cag tta ccg gat gga ccg gtg atc atg agc     2688
Val Glu Gln Asn Asn Tyr Gln Leu Pro Asp Gly Pro Val Ile Met Ser
                885                 890                 895

cct gat cgc ttg atg acc gcc ttc cac agg gag gtg att atg agg aag     2736
Pro Asp Arg Leu Met Thr Ala Phe His Arg Glu Val Ile Met Arg Lys
            900                 905                 910

cca gaa gaa ttc aag atg gca tgt ctg cgt gac att cgg agg ctg ttt     2784
Pro Glu Glu Phe Lys Met Ala Cys Leu Arg Asp Ile Arg Arg Leu Phe
        915                 920                 925

gga gat cgc aac ccc ttc tat gcc ggg ttt gga aac aga atc acg gac     2832
Gly Asp Arg Asn Pro Phe Tyr Ala Gly Phe Gly Asn Arg Ile Thr Asp
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gca ctg tcc tac agg agc gtt aat gtc ccc tca tct cgg ata ttt aca     2880
Ala Leu Ser Tyr Arg Ser Val Asn Val Pro Ser Ser Arg Ile Phe Thr
945                 950                 955                 960

att gat tcg gga ggt gaa gtc aag ctg gag ctc ctc agc agc tac aaa     2928
Ile Asp Ser Gly Gly Glu Val Lys Leu Glu Leu Leu Ser Ser Tyr Lys
                965                 970                 975

tca tca tat ctc gcg ttg aac gat ctc gtg aat gag atc ttt cca gga     2976
Ser Ser Tyr Leu Ala Leu Asn Asp Leu Val Asn Glu Ile Phe Pro Gly
            980                 985                 990

aaa aga cag gca ccc gag ttc aat  gac tgg aac ttt tgg  cgg gcg ccc   3024
Lys Arg Gln Ala Pro Glu Phe Asn  Asp Trp Asn Phe Trp  Arg Ala Pro
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ttg cca  gat atc gag ctt cca  gtt gcg ccg tct cat  caa tac gcc      3069
Leu Pro  Asp Ile Glu Leu Pro  Val Ala Pro Ser His  Gln Tyr Ala
    1010                1015                1020

cct aca  gcg gtg ccg ggc gag  tac aat gca caa gga  tat tct gca      3114
Pro Thr  Ala Val Pro Gly Glu  Tyr Asn Ala Gln Gly  Tyr Ser Ala
    1025                1030                1035

ggt cct  ggc cgg ttg gga gtg  ata cgg agc ctt acc  agt tcc ctc      3159
Gly Pro  Gly Arg Leu Gly Val  Ile Arg Ser Leu Thr  Ser Ser Leu
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acc tca  gca gga ccg ctc aag  acg agg acc gct atc  cca att ttt      3204
Thr Ser  Ala Gly Pro Leu Lys  Thr Arg Thr Ala Ile  Pro Ile Phe
    1055                1060                1065

acc tca  aat tcg ccc cct cct  ccg aat tcc tac cca  tcg gcg atg      3249
Thr Ser  Asn Ser Pro Pro Pro  Pro Asn Ser Tyr Pro  Ser Ala Met
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aag ccc  cat gca ccg cat cag  tcc caa cca gcc tcc  tcc tcg cct      3294
Lys Pro  His Ala Pro His Gln  Ser Gln Pro Ala Ser  Ser Ser Pro
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caa ccc  ccc gca tca gcg ccg  tca gga ctg cag atc  gct gat agg      3339
Gln Pro  Pro Ala Ser Ala Pro  Ser Gly Leu Gln Ile  Ala Asp Arg
    1100                1105                1110

acc cgt  cga ctc tcg ctg tcg  ttg atg cga tat agc  agc cat tca      3384
Thr Arg  Arg Leu Ser Leu Ser  Leu Met Arg Tyr Ser  Ser His Ser
    1115                1120                1125

gct ccc  acg tcc gcg cca gtt  ttg aga act ttg acc  gac agt tcc      3429
Ala Pro  Thr Ser Ala Pro Val  Leu Arg Thr Leu Thr  Asp Ser Ser
    1130                1135                1140

gag ccc  aat gtc ggc att gac  agc ggt gat gca ggc  gct ctc tct      3474
Glu Pro  Asn Val Gly Ile Asp  Ser Gly Asp Ala Gly  Ala Leu Ser
    1145                1150                1155

gag ggg  aat cag gca ggt tta  gag cca aat cgc tca  cct cac ttg      3519
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    1160                1165                1170
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aag aga  aag gca tct ggt ttc  tcg gtc tca ccg ccc  cag ctt gcc      3609
Lys Arg  Lys Ala Ser Gly Phe  Ser Val Ser Pro Pro  Gln Leu Ala
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agt cga  cta agt gag act gta  atg cct ttt ctt cgc  cga cga gca      3654
Ser Arg  Leu Ser Glu Thr Val  Met Pro Phe Leu Arg  Arg Arg Ala
    1205                 1210                 1215

tcc aag  ttg gag cag ggg cag  gag cag cag cag gaa  cag cag cag      3699
Ser Lys  Leu Glu Gln Gly Gln  Glu Gln Gln Gln Glu  Gln Gln Gln
    1220                 1225                 1230

gaa cag  gaa cag gaa cga gag  cat gat gtc cag ctg  ggt gca gca      3744
Glu Gln  Glu Gln Glu Arg Glu  His Asp Val Gln Leu  Gly Ala Ala
    1235                 1240                 1245

gct gaa  ggg gag cag ctt gct  tac act cga gag tac  ggg gaa gaa      3789
Ala Glu  Gly Glu Gln Leu Ala  Tyr Thr Arg Glu Tyr  Gly Glu Glu
    1250                 1255                 1260

gaa gcc  gct gct gga tat ctg  gcg gag gac cat gaa  ctc gga gag      3834
Glu Ala  Ala Ala Gly Tyr Leu  Ala Glu Asp His Glu  Leu Gly Glu
    1265                 1270                 1275

gat gaa  gag gat gaa gga gaa  gga gca gat gga tat  gtt ggt tat      3879
Asp Glu  Glu Asp Glu Gly Glu  Gly Ala Asp Gly Tyr  Val Gly Tyr
    1280                 1285                 1290

tct gga  gaa gag gat gaa ggt  ctg gaa gaa gat cag  ctc gag ggt      3924
Ser Gly  Glu Glu Asp Glu Gly  Leu Glu Glu Asp Gln  Leu Glu Gly
    1295                 1300                 1305

gag gaa  gac gag gat gag gat  gac gat gat gta gag  ctc aac att      3969
Glu Glu  Asp Glu Asp Glu Asp  Asp Asp Asp Val Glu  Leu Asn Ile
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            20                  25                  30

Glu Gln Ala Asp Gly Glu Leu Ala Cys Ser Pro Phe His Val Arg Phe
        35                  40                  45

Gly Lys Leu Ser Ile Leu Arg Pro Gln Glu Lys Val Val Glu Val Thr
    50                  55                  60

Val Asn Gly Arg Val Val Asp Phe Pro Met Lys Val Gly Asp Ala Gly
65                  70                  75                  80

Glu Ala Phe Phe Val Phe Glu Thr Glu Gln Asp Val Pro Glu Glu Phe
                85                  90                  95

Ala Thr Ser Pro Leu Ala Gly Pro Asn Thr Asp Lys Val Glu Glu Asp
            100                 105                 110

Ile Asp Tyr Leu Asp Leu Ala Glu Gly His Ser Thr Val Thr Tyr Pro
        115                 120                 125

Pro Asp Asp Ile Val Leu Asp Ala Gly Tyr Val Ser Ala His Ser Gly
    130                 135                 140
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His Gly Ser Glu Phe Glu Glu Asp Glu Arg Ala Asp Leu Ser Pro Glu
145                 150                 155                 160

Phe Asp Lys Lys Pro Asp Tyr Ala Ser Ala Val Lys Tyr Gly Gly Thr
                165                 170                 175

Asn Gly Gln Gly Arg His Leu Gly Ser Ala Asn Glu Ala Thr Thr Ser
            180                 185                 190

Val His Ala Phe Met Glu Arg Gln Val Gln Arg Trp Ser Leu Thr Met
        195                 200                 205

Ser Leu Pro Pro Ser Pro Val Leu Lys Ser Arg Asp Ile Met Glu Asn
    210                 215                 220

Phe Gln Pro Ile Asp Ser Ala Gly Pro Phe Asp Asn Ser Arg Glu Asp
225                 230                 235                 240

Ser Gly Arg Leu Leu Ala Pro Glu Thr Ile Ala Val Ser Asn Gly Gly
                245                 250                 255

Ser Ser Gly Ser Leu Phe His Pro Lys Glu Gly Met Ile Met Asp Met
            260                 265                 270

Thr Gly Tyr Lys Thr Glu Asp Ser Asp Leu Asn Ser Asp Ala Ser Asp
        275                 280                 285

Glu His Asp Val Gly Met Ala Gly Ala Leu Asn Gly Arg His Arg Arg
    290                 295                 300

Lys Arg Ala Ala Arg Arg Lys Arg Arg Gly Pro Val His Gly Val Asn
305                 310                 315                 320

Ser Gln Asp Asn Leu Ala Thr Glu Thr Pro Ser Ile Thr Ala His Val
                325                 330                 335

Leu Ser Ser Leu Asp Pro Arg Leu Pro Leu Arg Pro Thr Ala Arg Pro
            340                 345                 350

Ala Leu Arg Pro Lys Ala Asn Asn Gly Leu Gly Thr Leu Pro Asn Arg
        355                 360                 365

Arg Ser Ser Ser Met Pro Asn Leu Lys Asp Phe Val Gly Glu Asn Asn
    370                 375                 380

Ser Leu Ser Pro Ser Val Pro Ala Ile Met Arg Arg Phe Pro Ser Lys
385                 390                 395                 400

Thr Leu Asn Ser Lys Phe Ser Ala Arg Ser Asp Ile Lys Asp Gly Thr
                405                 410                 415

Ser Ser Ser Ser Ser Val Ala Ser Ser Pro Pro Pro Ser Val Ala Asn
            420                 425                 430

Gln Gln Ser Pro Lys Asn Arg His His His His His His His Lys Glu
        435                 440                 445

His Thr Glu Gly Ser His Pro Arg Arg His Ser His Lys Pro Ser Gln
    450                 455                 460

Gln Val Gln Val Lys Lys Pro Pro Pro Arg Ser Asn Pro Ala Val Asn
465                 470                 475                 480

Ala Leu Ser Asp Thr Glu Leu Glu Tyr Gln Thr Pro Arg Thr Thr Ala
                485                 490                 495

Ala Thr Gln Glu Ser Glu Trp Ser Trp Gly Trp Gly Ser Leu Pro Val
            500                 505                 510

Lys Asn Asp Gly Leu Gly Thr Gly Glu Ala Asp His Lys Glu His His
        515                 520                 525

Ser Ser His Pro Ser Ile Asp Ile Pro Ala Pro Arg Lys Pro Val Leu
    530                 535                 540

Asn Glu Met Glu Ile Asp Gly Thr Val Tyr Arg Leu Ala Ile Ser Leu
545                 550                 555                 560
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Cys Pro Gly Asp Glu Phe Gly Lys Asp Leu Glu Ala Ser Glu Ala Leu
                565                 570                 575

Phe Ala Thr Asn Gln Val Ser Phe Asp Glu Phe Ala Lys Asp Pro Leu
            580                 585                 590

Lys Thr Leu Asn Asn Lys Asn Leu Val Cys Leu Ile Asn Asp Arg Tyr
        595                 600                 605

Phe Thr Trp Thr Ala Ala Gly Pro Tyr Leu Ser Ser Leu Met Leu Phe
    610                 615                 620

Arg Lys Pro Leu Ser Asp Glu Thr Leu His Gln Leu Ser Ala Lys Asp
625                 630                 635                 640

Ser Arg His Leu Ser Asp Arg Leu Ala Val Gln Asp Glu Pro Pro Thr
                645                 650                 655

Arg Phe Gly Ala Leu Ser Arg Trp Leu Arg Gly Ser Gln Thr Ser Ser
            660                 665                 670

Gln Leu Ser Ala Met Glu Gln Gly Gln Arg Gln Arg Thr Pro Ser Thr
        675                 680                 685

Asn Asp Ala Leu Gln Pro Ala Gln Leu Glu Glu Ser Gln Ala Leu Gln
    690                 695                 700

Ser Val Lys Val Glu Ser Ile Lys His Thr Ser Gly Ser His Ser Ser
705                 710                 715                 720

Leu Leu Arg Ala Pro Lys Pro Met Thr Arg Ser Thr Ser Leu Pro Ile
                725                 730                 735

Asp Glu Gly Ile Ala Gly Ser Ile Ser Asp Glu Tyr Ala Gly Ser Ser
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Pro Pro Thr His Ser Ala Leu Lys Ser Ser Arg Arg Tyr Ala Lys Thr
        755                 760                 765

Leu Arg Leu Thr Ser Glu Gln Leu Lys Ser Leu Asn Leu Lys Lys Gly
    770                 775                 780

Ala Asn Thr Leu Thr Phe Ser Val Thr Ser Ser Tyr Gln Gly Lys Ala
785                 790                 795                 800

Val Cys Ser Ala Lys Leu Phe Leu Trp Asp His Asp Tyr Gln Val Val
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Ile Ser Asp Ile Asp Gly Thr Ile Thr Lys Ser Asp Ala Leu Gly His
            820                 825                 830

Ile Phe Thr Met Ala Gly Lys Asp Trp Thr His Ser Gly Val Ala Lys
        835                 840                 845

Leu Tyr Thr Asp Ile Val Asn Asn Gly Tyr His Ile Leu Tyr Leu Thr
    850                 855                 860

Ser Arg Ala Ile Gly Gln Ala Asp Tyr Thr Arg Lys Tyr Leu Lys Asn
865                 870                 875                 880

Val Glu Gln Asn Asn Tyr Gln Leu Pro Asp Gly Pro Val Ile Met Ser
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Pro Asp Arg Leu Met Thr Ala Phe His Arg Glu Val Ile Met Arg Lys
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Pro Glu Glu Phe Lys Met Ala Cys Leu Arg Asp Ile Arg Arg Leu Phe
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Gly Asp Arg Asn Pro Phe Tyr Ala Gly Phe Gly Asn Arg Ile Thr Asp
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Ala Leu Ser Tyr Arg Ser Val Asn Val Pro Ser Ser Arg Ile Phe Thr
945                 950                 955                 960

Ile Asp Ser Gly Gly Glu Val Lys Leu Glu Leu Leu Ser Ser Tyr Lys
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Ser Ser Tyr Leu Ala Leu Asn Asp Leu Val Asn Glu Ile Phe Pro Gly
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Pro Thr  Ala Val Pro Gly Glu  Tyr Asn Ala Gln Gly  Tyr Ser Ala
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Gly Pro  Gly Arg Leu Gly Val  Ile Arg Ser Leu Thr  Ser Ser Leu
    1040                1045                1050

Thr Ser  Ala Gly Pro Leu Lys  Thr Arg Thr Ala Ile  Pro Ile Phe
    1055                1060                1065

Thr Ser  Asn Ser Pro Pro Pro  Pro Asn Ser Tyr Pro  Ser Ala Met
    1070                1075                1080

Lys Pro  His Ala Pro His Gln  Ser Gln Pro Ala Ser  Ser Ser Pro
    1085                1090                1095

Gln Pro  Pro Ala Ser Ala Pro  Ser Gly Leu Gln Ile  Ala Asp Arg
    1100                1105                1110

Thr Arg  Arg Leu Ser Leu Ser  Leu Met Arg Tyr Ser  Ser His Ser
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Ala Pro  Thr Ser Ala Pro Val  Leu Arg Thr Leu Thr  Asp Ser Ser
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Glu Pro  Asn Val Gly Ile Asp  Ser Gly Asp Ala Gly  Ala Leu Ser
    1145                1150                1155

Glu Gly  Asn Gln Ala Gly Leu  Glu Pro Asn Arg Ser  Pro His Leu
    1160                1165                1170

Gly Ser  Asn Thr Asp Gly Val  Phe Pro Leu Asp Val  Pro Val Val
    1175                1180                1185

Lys Arg  Lys Ala Ser Gly Phe  Ser Val Ser Pro Pro  Gln Leu Ala
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Ser Arg  Leu Ser Glu Thr Val  Met Pro Phe Leu Arg  Arg Arg Ala
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Ser Lys  Leu Glu Gln Gly Gln  Glu Gln Gln Gln Glu  Gln Gln Gln
    1220                1225                1230

Glu Gln  Glu Gln Glu Arg Glu  His Asp Val Gln Leu  Gly Ala Ala
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Ala Glu  Gly Glu Gln Leu Ala  Tyr Thr Arg Glu Tyr  Gly Glu Glu
    1250                1255                1260

Glu Ala  Ala Ala Gly Tyr Leu  Ala Glu Asp His Glu  Leu Gly Glu
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Asp Glu  Glu Asp Glu Gly Glu  Gly Ala Asp Gly Tyr  Val Gly Tyr
    1280                1285                1290

Ser Gly  Glu Glu Asp Glu Gly  Leu Glu Glu Asp Gln  Leu Glu Gly
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<212> TYPE: DNA
<213> ORGANISM: Mortierella alpina

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<212> TYPE: DNA
<213> ORGANISM: Mortierella alpina

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<211> LENGTH: 5034
<212> TYPE: DNA
<213> ORGANISM: Mortierella alpina

<400> SEQUENCE: 5

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caagggcaaa gacaacgtac tcccagtacc aacgatgcct tgcagcctgc tcagttagag 2760

gaggtacatg aaatcctctt ttattcaaaa agccccgaga tgcaatagta caaccagtta 2820

ctgacaacac ctcggtatcg ctgtagagtc aagctttaca gagcgtgaaa gtcgaatcga 2880

ttaagcacac ttccggatca cattcatcac ttctacgcgc tcctaaacca atgactcgta 2940

gcacctctct gccgatcgac gaagggatcg ccgggtctat atcagacgag tacgctggaa 3000

gctcgcctcc gacacattct gcgctcaaga gcagtagacg gtatgcgaaa acgcttcgct 3060

tgacatctga acagttggta cgtgcctaca accggatagc gtattgaatt gcgcgtgtac 3120

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cagcagcatt gaaatctcac acggcattgt ccgcttctga aatagaaatc actaaatttg 3180

aaaaaaggcg ccaatacatt gacgttttca gtaacgtcaa gttatcaagg caaagcagtt 3240

tgttccgcca aattgtttct gtgggaccat gactaccaag tcgtcatatc ggacattgat 3300

ggcacgatta caaagtcgga cgctctcgga cacatcttta ccatggcagg aaaggattgg 3360

acccattcgg gtgtcgccaa actttacacg gacatcgtca acaatgggta tcatattttg 3420

tacttgacct caagggccat tggacaggca gactacacac gaaagtacct caagaacgtg 3480

gagcaaaata actaccagtt accggatgga ccggtgatca tgagccctga tcgcttgatg 3540

accgccttcc acaggtcagc agtgttcact gtggcgcata ggcttcgtag ggatgggaca 3600

tcttgctttg aatgcttact aacaaccatt tgcgttaacg ttttagggag gtgattatga 3660

ggaagccaga agaattcaag atggcatgtc tgcgtgacat tcggaggctg tttggagatc 3720

gcaacccctt ctatgccggg tttggaaaca gaatcacgga cgcactgtcc tacaggagcg 3780

ttaatgtccc ctcatctcgg atatttacaa ttgattcggg aggtgaagtc aagctggagc 3840

tcctcagcag ctacaaatca tcgtgagtac ccttcactgc acttgctttt ccactggtgg 3900

cgtccatcca gtctttgttg gcgaaacatg gatttaggac ctgaccattt ttgtctcttt 3960

gctgatctac ttgacacaag atatctcgcg ttgaacgatc tcgtgaatga gatctttcca 4020

ggaaaaagac aggcacccga gttcaatgac tggaactttt ggcgggcgcc cttgccagat 4080

atcgagcttc cagttgcgcc gtctcatcaa tacgccccta cagcggtgcc gggcgagtac 4140

aatgcacaag gatattctgc aggtcctggc cggttgggag tgatacggag ccttaccagt 4200

tccctcacct cagcaggacc gctcaagacg aggaccgcta tcccaatttt tacctcaaat 4260

tcgccccctc ctccgaattc ctacccatcg gcgatgaagc cccatgcacc gcatcagtcc 4320

caaccagcct cctcctcgcc tcaacccccc gcatcagcgc cgtcaggact gcagatcgct 4380

gataggaccc gtcgactctc gctgtcgttg atgcgatata gcagccattc agctcccacg 4440

tccgcgccag ttttgagaac tttgaccgac agttccgagc ccaatgtcgg cattgacagc 4500

ggtgatgcag gcgctctctc tgaggggaat caggcaggtt tagagccaaa tcgctcacct 4560

cacttgggat ccaacactga tggcgttttc ccactggacg ttcctgttgt gaagagaaag 4620

gcatctggtt tctcggtctc accgccccag cttgccagtc gactaagtga gactgtaatg 4680

ccttttcttc gccgacgagc atccaagttg gagcaggggc aggagcagca gcaggaacag 4740

cagcaggaac aggaacagga acgagagcat gatgtccagc tgggtgcagc agctgaaggg 4800

gagcagcttg cttacactcg agagtacggg gaagaagaag ccgctgctgg atatctggcg 4860

gaggaccatg aactcggaga ggatgaagag gatgaaggag aaggagcaga tggatatgtt 4920

ggttattctg gagaagagga tgaaggtctg gaagaagatc agctcgaggg tgaggaagac 4980

gaggatgagg atgacgatga tgtagagctc aacattgacg ctccgttcct atga 5034

<210> SEQ ID NO 6
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<212> TYPE: DNA
<213> ORGANISM: Mortierella alpina
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (1)..(3717)
<223> OTHER INFORMATION:

<400> SEQUENCE: 6

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Met Tyr Ser Val Gly Asn Phe Phe Ser Thr Val Thr Lys Phe Tyr Asn

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1               5                  10                  15

gag atc aac ccc gcc acc ctc tcc ggc gca atc gac atc atc gtc gtc       96
Glu Ile Asn Pro Ala Thr Leu Ser Gly Ala Ile Asp Ile Ile Val Val
            20                  25                  30

cag cag gcc aac ggc gac ctt gca tgc tct ccc ttc cac gtg cgt ttc      144
Gln Gln Ala Asn Gly Asp Leu Ala Cys Ser Pro Phe His Val Arg Phe
        35                  40                  45

ggc aaa ctc agc gtc ctc cgg ccg cag gag aag gtc gtc gag gtt cgg      192
Gly Lys Leu Ser Val Leu Arg Pro Gln Glu Lys Val Val Glu Val Arg
    50                  55                  60

gtc aat ggc gaa gtc atc gcc ttc ccc atg aag gtc ggc gac gca gga      240
Val Asn Gly Glu Val Ile Ala Phe Pro Met Lys Val Gly Asp Ala Gly
65                  70                  75                  80

gag gcc ttc ttt gtg ctc gag acc gac gac tat gtg ccg gat gag ttt      288
Glu Ala Phe Phe Val Leu Glu Thr Asp Asp Tyr Val Pro Asp Glu Phe
                85                  90                  95

gcc aca tcg cct atc gct ggt ccg agt gac gaa gcc gac ctc gcc cct      336
Ala Thr Ser Pro Ile Ala Gly Pro Ser Asp Glu Ala Asp Leu Ala Pro
            100                 105                 110

gtt gac tac ttt gac ctg aac ggc cat ccc cac ggg tct cag gac cag      384
Val Asp Tyr Phe Asp Leu Asn Gly His Pro His Gly Ser Gln Asp Gln
        115                 120                 125

aaa cgg agg cag cat cag cag caa cag gtg ctg gag ggc atg agc gga      432
Lys Arg Arg Gln His Gln Gln Gln Gln Val Leu Glu Gly Met Ser Gly
    130                 135                 140

cag tat cct caa gga aca gaa gac gat gct cct ctt gac aac ggc tat      480
Gln Tyr Pro Gln Gly Thr Glu Asp Asp Ala Pro Leu Asp Asn Gly Tyr
145                 150                 155                 160

gtg agc gct gct agt ggc cat ggc tct gct ttt gaa gag agc ttg aag      528
Val Ser Ala Ala Ser Gly His Gly Ser Ala Phe Glu Glu Ser Leu Lys
                165                 170                 175

gac gac agc gat cac gag tcg gtc ttc tcg gcc aca tcc cca gga tca      576
Asp Asp Ser Asp His Glu Ser Val Phe Ser Ala Thr Ser Pro Gly Ser
            180                 185                 190

gca gaa cgg atc gcc gcc gat tct aat act aag gac aca gca ctc gac      624
Ala Glu Arg Ile Ala Ala Asp Ser Asn Thr Lys Asp Thr Ala Leu Asp
        195                 200                 205

ttg cct gga tcc ttt ggc cca acg gta gtg act aat acc atc aaa aac      672
Leu Pro Gly Ser Phe Gly Pro Thr Val Val Thr Asn Thr Ile Lys Asn
    210                 215                 220

aag gac agc atc aac ttt cca gtt gat gcc atc ttt cct aca gtt gca      720
Lys Asp Ser Ile Asn Phe Pro Val Asp Ala Ile Phe Pro Thr Val Ala
225                 230                 235                 240

cac gag gaa cag gac atg gct ctg atc aaa gat caa cag ggc tct cga      768
His Glu Glu Gln Asp Met Ala Leu Ile Lys Asp Gln Gln Gly Ser Arg
                245                 250                 255

tcc agc cgt cgc aga agt gag gtc cta ttc gat atg aca gga tac aag      816
Ser Ser Arg Arg Arg Ser Glu Val Leu Phe Asp Met Thr Gly Tyr Lys
            260                 265                 270

acc gac tca tgc tcg gac tcg tcg gat gat gag gat ggc ttg cct cgt      864
Thr Asp Ser Cys Ser Asp Ser Ser Asp Asp Glu Asp Gly Leu Pro Arg
        275                 280                 285

ggc att cta tcg gat agt gag cgt cac ggt cgt agc acg cgt aag aag      912
Gly Ile Leu Ser Asp Ser Glu Arg His Gly Arg Ser Thr Arg Lys Lys
    290                 295                 300

ttc agg agg agc aag tcg cac ctt tca atg gag cag agg cac caa ttg      960
Phe Arg Arg Ser Lys Ser His Leu Ser Met Glu Gln Arg His Gln Leu
305                 310                 315                 320

ctg gag gac att aaa caa gga gcg ttc ctg aag ccc gag gaa agc ctt     1008
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Leu Glu Asp Ile Lys Gln Gly Ala Phe Leu Lys Pro Glu Glu Ser Leu
                325                 330                 335

gca aac aca cag att gaa cgt caa aca tcc cgg gca agt agg aaa aca     1056
Ala Asn Thr Gln Ile Glu Arg Gln Thr Ser Arg Ala Ser Arg Lys Thr
            340                 345                 350

aag agg gca agc att cca agt gca tgg caa gga cga agg aac agg aag     1104
Lys Arg Ala Ser Ile Pro Ser Ala Trp Gln Gly Arg Arg Asn Arg Lys
        355                 360                 365

aga gcc aac agc atg cct gct atc ggt gaa cca gac ttg gca ttt cct     1152
Arg Ala Asn Ser Met Pro Ala Ile Gly Glu Pro Asp Leu Ala Phe Pro
    370                 375                 380

gcc tat gtg gct cgc cga cct aac cat cgt cgc gat gct caa gca aac     1200
Ala Tyr Val Ala Arg Arg Pro Asn His Arg Arg Asp Ala Gln Ala Asn
385                 390                 395                 400

cag acg gat gtt gca atg gac gac aag ccc aag ccc aag cgc act gct     1248
Gln Thr Asp Val Ala Met Asp Asp Lys Pro Lys Pro Lys Arg Thr Ala
                405                 410                 415

cgg ccc agc gtt atg agc gat acg gag atg gag tat gaa tcc aac aat     1296
Arg Pro Ser Val Met Ser Asp Thr Glu Met Glu Tyr Glu Ser Asn Asn
            420                 425                 430

gtc cct gca tct acc cag ggt aaa gag tgg acc tgg gga tgg gga acg     1344
Val Pro Ala Ser Thr Gln Gly Lys Glu Trp Thr Trp Gly Trp Gly Thr
        435                 440                 445

ctg cct gtc aaa cag gat aac cct gat gaa gag gat gag atc aag gaa     1392
Leu Pro Val Lys Gln Asp Asn Pro Asp Glu Glu Asp Glu Ile Lys Glu
    450                 455                 460

caa att acg gaa gaa aag gcg ccc gaa gtt cct gtg gag att gag gca     1440
Gln Ile Thr Glu Glu Lys Ala Pro Glu Val Pro Val Glu Ile Glu Ala
465                 470                 475                 480

aag gag ttt cag atg gga tca aca aaa tgc cgc gta gcg ctc agt ctc     1488
Lys Glu Phe Gln Met Gly Ser Thr Lys Cys Arg Val Ala Leu Ser Leu
                485                 490                 495

tgc gga gag gat gac ttt gga aag gac att gtt gct agc cac aag gct     1536
Cys Gly Glu Asp Asp Phe Gly Lys Asp Ile Val Ala Ser His Lys Ala
            500                 505                 510

ttt caa aga gcc cag ttg acc ttt gag gca ttc tcc aaa gat ccc gcg     1584
Phe Gln Arg Ala Gln Leu Thr Phe Glu Ala Phe Ser Lys Asp Pro Ala
        515                 520                 525

gca att ctg gcc gac aag aga ctt gtg tgt tac atg gat ggg cgg ttt     1632
Ala Ile Leu Ala Asp Lys Arg Leu Val Cys Tyr Met Asp Gly Arg Phe
    530                 535                 540

tat tcg tgg agt aat gcc gtt cct cag ctc gca gcc ctt ctc ttc ttc     1680
Tyr Ser Trp Ser Asn Ala Val Pro Gln Leu Ala Ala Leu Leu Phe Phe
545                 550                 555                 560

cac cag cct ctt tca gac gcg gcc tct gct ctc gac ctc aag gac caa     1728
His Gln Pro Leu Ser Asp Ala Ala Ser Ala Leu Asp Leu Lys Asp Gln
                565                 570                 575

aag gca cat gcg gcc gag gac aga ccg agc gcc acg cgt ttt ggc aca     1776
Lys Ala His Ala Ala Glu Asp Arg Pro Ser Ala Thr Arg Phe Gly Thr
            580                 585                 590

atc tcc aga tgg ttc agg aag gcg cct gca ggc agc gcg tcc ccc tct     1824
Ile Ser Arg Trp Phe Arg Lys Ala Pro Ala Gly Ser Ala Ser Pro Ser
        595                 600                 605

att gca gat atg gcc tca gca tcc tcg aca acc ctt gca ggt ggt gag     1872
Ile Ala Asp Met Ala Ser Ala Ser Ser Thr Thr Leu Ala Gly Gly Glu
    610                 615                 620

acc gcc gct gtc gct gtg gga tca gat gac gac gag ccc ttg cac aac     1920
Thr Ala Ala Val Ala Val Gly Ser Asp Asp Asp Glu Pro Leu His Asn
625                 630                 635                 640
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aag gcc ctg cgt agc aaa tcc ctg ccc cca ctg gag act ggc cgg acc     1968
Lys Ala Leu Arg Ser Lys Ser Leu Pro Pro Leu Glu Thr Gly Arg Thr
                645                 650                 655

gac gac cac agt cag agc cat gtc gct gta cct gcg ctt tcg gag aaa     2016
Asp Asp His Ser Gln Ser His Val Ala Val Pro Ala Leu Ser Glu Lys
            660                 665                 670

gca gcg gac ggt gtc cca gat cag aag cgc tat gcc aag acg ctg cgg     2064
Ala Ala Asp Gly Val Pro Asp Gln Lys Arg Tyr Ala Lys Thr Leu Arg
        675                 680                 685

ctc acc tcg gaa cag ctt caa tcc ttg ggt ttg aaa aag ggc gcc aac     2112
Leu Thr Ser Glu Gln Leu Gln Ser Leu Gly Leu Lys Lys Gly Ala Asn
    690                 695                 700

acg gtc tcg ttc tca gtg aca tcg tcc tac cag gga act gca act tgt     2160
Thr Val Ser Phe Ser Val Thr Ser Ser Tyr Gln Gly Thr Ala Thr Cys
705                 710                 715                 720

gta gcc aag atc ttt ttg tgg gat tac gac tcc cag gtg gtg atc tcg     2208
Val Ala Lys Ile Phe Leu Trp Asp Tyr Asp Ser Gln Val Val Ile Ser
                725                 730                 735

gat att gat ggt aca atc aca aag tca gat gcc ctc ggc cac att ttt     2256
Asp Ile Asp Gly Thr Ile Thr Lys Ser Asp Ala Leu Gly His Ile Phe
            740                 745                 750

gcc atg gcc ggt cgc gac tgg acg cat ctc ggt gtc gcc aag ctg ttc     2304
Ala Met Ala Gly Arg Asp Trp Thr His Leu Gly Val Ala Lys Leu Phe
        755                 760                 765

aca gat att cgc agc aac gga tat cac atc ctg tac ctg acc tcc cga     2352
Thr Asp Ile Arg Ser Asn Gly Tyr His Ile Leu Tyr Leu Thr Ser Arg
    770                 775                 780

gcc att ggc cag gca gac tac aca cgc aag tat ctt cag aag gtc gag     2400
Ala Ile Gly Gln Ala Asp Tyr Thr Arg Lys Tyr Leu Gln Lys Val Glu
785                 790                 795                 800

caa aac agt tac cag ctc ccg gat ggc cct gtc atc atg agt cca gac     2448
Gln Asn Ser Tyr Gln Leu Pro Asp Gly Pro Val Ile Met Ser Pro Asp
                805                 810                 815

cgt ctg ttc tct gcc ttc cat cgt gag gtg att atc cgg aaa cca gag     2496
Arg Leu Phe Ser Ala Phe His Arg Glu Val Ile Ile Arg Lys Pro Glu
            820                 825                 830

gtg ttc aag atg gcg tgt ctg cgt gat gtg aag aag ctg ttt ggg gac     2544
Val Phe Lys Met Ala Cys Leu Arg Asp Val Lys Lys Leu Phe Gly Asp
        835                 840                 845

agg aac ccg ttc tat gct gga ttt gga aac cgg atc acg gac gcc ctc     2592
Arg Asn Pro Phe Tyr Ala Gly Phe Gly Asn Arg Ile Thr Asp Ala Leu
    850                 855                 860

tcc tac cgc agt gtc aac gtt cca ccc tcc cga atc ttc acc att gac     2640
Ser Tyr Arg Ser Val Asn Val Pro Pro Ser Arg Ile Phe Thr Ile Asp
865                 870                 875                 880

tct tat ggt gag gtg aag ttg gag ctg ctc agt gct ttc aag tct tca     2688
Ser Tyr Gly Glu Val Lys Leu Glu Leu Leu Ser Ala Phe Lys Ser Ser
                885                 890                 895

tac ttg gct ttg aat gac ctc gtc aat gag atc ttc cca gga caa cga     2736
Tyr Leu Ala Leu Asn Asp Leu Val Asn Glu Ile Phe Pro Gly Gln Arg
            900                 905                 910

gtt gca ccc gag ttc aac gac tgg aac ttt tgg aaa tcg gat tta cca     2784
Val Ala Pro Glu Phe Asn Asp Trp Asn Phe Trp Lys Ser Asp Leu Pro
        915                 920                 925

cgg att gat ctc cct gat ctc ccc atc ccc aac aat aat tat aca tca     2832
Arg Ile Asp Leu Pro Asp Leu Pro Ile Pro Asn Asn Asn Tyr Thr Ser
    930                 935                 940

gga tct tcg aca tcg ctc ctc tca tcc acc act agc gtg gcc aag aag     2880
Gly Ser Ser Thr Ser Leu Leu Ser Ser Thr Thr Ser Val Ala Lys Lys
945                 950                 955                 960
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gtg gcg tct ttg acc agc tct tca tcg agc tcg aac ctt ctc cag cca     2928
Val Ala Ser Leu Thr Ser Ser Ser Ser Ser Ser Asn Leu Leu Gln Pro
               965                 970                 975

acg tcg ccc act agc cct acg gga gat ttc aag aac aag cgc ctg tct     2976
Thr Ser Pro Thr Ser Pro Thr Gly Asp Phe Lys Asn Lys Arg Leu Ser
           980                 985                 990

aat gac aga aac acg tat gcg ggc  gtc ctt tca gga cgt  cag gac aca   3024
Asn Asp Arg Asn Thr Tyr Ala Gly  Val Leu Ser Gly Arg  Gln Asp Thr
       995                 1000                1005

tgg acc  agc gat gat gaa tat  cag gat caa cag cag  cga ctg atc      3069
Trp Thr  Ser Asp Asp Glu Tyr  Gln Asp Gln Gln Gln  Arg Leu Ile
    1010                1015                1020

gcg ggt  gac tct gcg ccg tca  acg cca gga tca gag  ttg aag gca      3114
Ala Gly  Asp Ser Ala Pro Ser  Thr Pro Gly Ser Glu  Leu Lys Ala
    1025                1030                1035

gga cag  gag ctg aag gag gat  gca agg aag gca cga  tct ggc tcg      3159
Gly Gln  Glu Leu Lys Glu Asp  Ala Arg Lys Ala Arg  Ser Gly Ser
    1040                1045                1050

cca tcg  atg ctc tct gct ctt  gtt cca tcg cgg tta  atc cgc gca      3204
Pro Ser  Met Leu Ser Ala Leu  Val Pro Ser Arg Leu  Ile Arg Ala
    1055                1060                1065

gtg agg  agt ggc agc atc agc  agt cag acc aac cct  gtg ccc tcg      3249
Val Arg  Ser Gly Ser Ile Ser  Ser Gln Thr Asn Pro  Val Pro Ser
    1070                1075                1080

tcg atg  cgg agt tcg gtt aca  ccg cat tcg ccc gag  atg aaa ggg      3294
Ser Met  Arg Ser Ser Val Thr  Pro His Ser Pro Glu  Met Lys Gly
    1085                1090                1095

atc atc  ggg tcg ctg ccg tca  cca gtg tct tcg ttt  gag agc ggt      3339
Ile Ile  Gly Ser Leu Pro Ser  Pro Val Ser Ser Phe  Glu Ser Gly
    1100                1105                1110

gcg gat  gtg gtg cgt cgg atg  tcc att ccc tcg cct  cca ccg ttg      3384
Ala Asp  Val Val Arg Arg Met  Ser Ile Pro Ser Pro  Pro Pro Leu
    1115                1120                1125

gag ggg  ctg ctc cag acg gat  gag gag gtg gct cag  gca tcg agc      3429
Glu Gly  Leu Leu Gln Thr Asp  Glu Glu Val Ala Gln  Ala Ser Ser
    1130                1135                1140

aag gcg  ctg gcg ctt cag gga  tcg gac aca gca gat  ttg agc aga      3474
Lys Ala  Leu Ala Leu Gln Gly  Ser Asp Thr Ala Asp  Leu Ser Arg
    1145                1150                1155

gag agc  agt gtt cag gcc aag  agt gat gtg atg gac  gac ctt gtg      3519
Glu Ser  Ser Val Gln Ala Lys  Ser Asp Val Met Asp  Asp Leu Val
    1160                1165                1170

gcg gtc  aag gag gaa gag gag  gac gag acc gat cag  cag cgg ttg      3564
Ala Val  Lys Glu Glu Glu Glu  Asp Glu Thr Asp Gln  Gln Arg Leu
    1175                1180                1185

ctg gat  gca gcg tat gtg gat  gag tat gtg gat gag  gag gat gag      3609
Leu Asp  Ala Ala Tyr Val Asp  Glu Tyr Val Asp Glu  Glu Asp Glu
    1190                1195                1200

gag gga  tat gat gga tat gac  gag cag ggt gag gat  gag atg gac      3654
Glu Gly  Tyr Asp Gly Tyr Asp  Glu Gln Gly Glu Asp  Glu Met Asp
    1205                1210                1215

gag gag  gat gag gag gac gag  tat ctg gat gag att  gag gag act      3699
Glu Glu  Asp Glu Glu Asp Glu  Tyr Leu Asp Glu Ile  Glu Glu Thr
    1220                1225                1230

ctg gag  gag ccg ttc ctg                                        3717
Leu Glu  Glu Pro Phe Leu
    1235
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<210> SEQ ID NO 7

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<211> LENGTH: 1239
<212> TYPE: PRT
<213> ORGANISM: Mortierella alpina

<400> SEQUENCE: 7

Met Tyr Ser Val Gly Asn Phe Phe Ser Thr Val Thr Lys Phe Tyr Asn
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Glu Ile Asn Pro Ala Thr Leu Ser Gly Ala Ile Asp Ile Ile Val Val
20 25 30

Gln Gln Ala Asn Gly Asp Leu Ala Cys Ser Pro Phe His Val Arg Phe
35 40 45

Gly Lys Leu Ser Val Leu Arg Pro Gln Glu Lys Val Val Glu Val Arg
50 55 60

Val Asn Gly Glu Val Ile Ala Phe Pro Met Lys Val Gly Asp Ala Gly
65 70 75 80

Glu Ala Phe Phe Val Leu Glu Thr Asp Asp Tyr Val Pro Asp Glu Phe
85 90 95

Ala Thr Ser Pro Ile Ala Gly Pro Ser Asp Glu Ala Asp Leu Ala Pro
100 105 110

Val Asp Tyr Phe Asp Leu Asn Gly His Pro His Gly Ser Gln Asp Gln
115 120 125

Lys Arg Arg Gln His Gln Gln Gln Gln Val Leu Glu Gly Met Ser Gly
130 135 140

Gln Tyr Pro Gln Gly Thr Glu Asp Asp Ala Pro Leu Asp Asn Gly Tyr
145 150 155 160

Val Ser Ala Ala Ser Gly His Gly Ser Ala Phe Glu Glu Ser Leu Lys
165 170 175

Asp Asp Ser Asp His Glu Ser Val Phe Ser Ala Thr Ser Pro Gly Ser
180 185 190

Ala Glu Arg Ile Ala Ala Asp Ser Asn Thr Lys Asp Thr Ala Leu Asp
195 200 205

Leu Pro Gly Ser Phe Gly Pro Thr Val Val Thr Asn Thr Ile Lys Asn
210 215 220

Lys Asp Ser Ile Asn Phe Pro Val Asp Ala Ile Phe Pro Thr Val Ala
225 230 235 240

His Glu Glu Gln Asp Met Ala Leu Ile Lys Asp Gln Gln Gly Ser Arg
245 250 255

Ser Ser Arg Arg Arg Ser Glu Val Leu Phe Asp Met Thr Gly Tyr Lys
260 265 270

Thr Asp Ser Cys Ser Asp Ser Ser Asp Asp Glu Asp Gly Leu Pro Arg
275 280 285

Gly Ile Leu Ser Asp Ser Glu Arg His Gly Arg Ser Thr Arg Lys Lys
290 295 300

Phe Arg Arg Ser Lys Ser His Leu Ser Met Glu Gln Arg His Gln Leu
305 310 315 320

Leu Glu Asp Ile Lys Gln Gly Ala Phe Leu Lys Pro Glu Glu Ser Leu
325 330 335

Ala Asn Thr Gln Ile Glu Arg Gln Thr Ser Arg Ala Ser Arg Lys Thr
340 345 350

Lys Arg Ala Ser Ile Pro Ser Ala Trp Gln Gly Arg Arg Asn Arg Lys
355 360 365

Arg Ala Asn Ser Met Pro Ala Ile Gly Glu Pro Asp Leu Ala Phe Pro
370 375 380

Ala Tyr Val Ala Arg Arg Pro Asn His Arg Arg Asp Ala Gln Ala Asn

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385                 390                 395                 400

Gln Thr Asp Val Ala Met Asp Asp Lys Pro Lys Pro Lys Arg Thr Ala
                405                 410                 415

Arg Pro Ser Val Met Ser Asp Thr Glu Met Glu Tyr Glu Ser Asn Asn
            420                 425                 430

Val Pro Ala Ser Thr Gln Gly Lys Glu Trp Thr Trp Gly Trp Gly Thr
        435                 440                 445

Leu Pro Val Lys Gln Asp Asn Pro Asp Glu Glu Asp Glu Ile Lys Glu
    450                 455                 460

Gln Ile Thr Glu Glu Lys Ala Pro Glu Val Pro Val Glu Ile Glu Ala
465                 470                 475                 480

Lys Glu Phe Gln Met Gly Ser Thr Lys Cys Arg Val Ala Leu Ser Leu
                485                 490                 495

Cys Gly Glu Asp Asp Phe Gly Lys Asp Ile Val Ala Ser His Lys Ala
            500                 505                 510

Phe Gln Arg Ala Gln Leu Thr Phe Glu Ala Phe Ser Lys Asp Pro Ala
        515                 520                 525

Ala Ile Leu Ala Asp Lys Arg Leu Val Cys Tyr Met Asp Gly Arg Phe
    530                 535                 540

Tyr Ser Trp Ser Asn Ala Val Pro Gln Leu Ala Ala Leu Leu Phe Phe
545                 550                 555                 560

His Gln Pro Leu Ser Asp Ala Ala Ser Ala Leu Asp Leu Lys Asp Gln
                565                 570                 575

Lys Ala His Ala Ala Glu Asp Arg Pro Ser Ala Thr Arg Phe Gly Thr
            580                 585                 590

Ile Ser Arg Trp Phe Arg Lys Ala Pro Ala Gly Ser Ala Ser Pro Ser
        595                 600                 605

Ile Ala Asp Met Ala Ser Ala Ser Ser Thr Thr Leu Ala Gly Gly Glu
    610                 615                 620

Thr Ala Ala Val Ala Val Gly Ser Asp Asp Asp Glu Pro Leu His Asn
625                 630                 635                 640

Lys Ala Leu Arg Ser Lys Ser Leu Pro Pro Leu Glu Thr Gly Arg Thr
                645                 650                 655

Asp Asp His Ser Gln Ser His Val Ala Val Pro Ala Leu Ser Glu Lys
            660                 665                 670

Ala Ala Asp Gly Val Pro Asp Gln Lys Arg Tyr Ala Lys Thr Leu Arg
        675                 680                 685

Leu Thr Ser Glu Gln Leu Gln Ser Leu Gly Leu Lys Lys Gly Ala Asn
    690                 695                 700

Thr Val Ser Phe Ser Val Thr Ser Ser Tyr Gln Gly Thr Ala Thr Cys
705                 710                 715                 720

Val Ala Lys Ile Phe Leu Trp Asp Tyr Asp Ser Gln Val Val Ile Ser
                725                 730                 735

Asp Ile Asp Gly Thr Ile Thr Lys Ser Asp Ala Leu Gly His Ile Phe
            740                 745                 750

Ala Met Ala Gly Arg Asp Trp Thr His Leu Gly Val Ala Lys Leu Phe
        755                 760                 765

Thr Asp Ile Arg Ser Asn Gly Tyr His Ile Leu Tyr Leu Thr Ser Arg
    770                 775                 780

Ala Ile Gly Gln Ala Asp Tyr Thr Arg Lys Tyr Leu Gln Lys Val Glu
785                 790                 795                 800

Gln Asn Ser Tyr Gln Leu Pro Asp Gly Pro Val Ile Met Ser Pro Asp
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Arg Leu Phe Ser Ala Phe His Arg Glu Val Ile Ile Arg Lys Pro Glu
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Val Phe Lys Met Ala Cys Leu Arg Asp Val Lys Lys Leu Phe Gly Asp
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Arg Asn Pro Phe Tyr Ala Gly Phe Gly Asn Arg Ile Thr Asp Ala Leu
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Ser Tyr Arg Ser Val Asn Val Pro Pro Ser Arg Ile Phe Thr Ile Asp
865                 870                 875                 880

Ser Tyr Gly Glu Val Lys Leu Glu Leu Leu Ser Ala Phe Lys Ser Ser
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Tyr Leu Ala Leu Asn Asp Leu Val Asn Glu Ile Phe Pro Gly Gln Arg
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Val Ala Pro Glu Phe Asn Asp Trp Asn Phe Trp Lys Ser Asp Leu Pro
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Arg Ile Asp Leu Pro Asp Leu Pro Ile Pro Asn Asn Asn Tyr Thr Ser
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Gly Ser Ser Thr Ser Leu Leu Ser Ser Thr Thr Ser Val Ala Lys Lys
945                 950                 955                 960

Val Ala Ser Leu Thr Ser Ser Ser Ser Ser Ser Asn Leu Leu Gln Pro
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Thr Ser Pro Thr Ser Pro Thr Gly Asp Phe Lys Asn Lys Arg Leu Ser
            980                 985                 990

Asn Asp Arg Asn Thr Tyr Ala Gly  Val Leu Ser Gly Arg  Gln Asp Thr
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Trp Thr  Ser Asp Asp Glu Tyr  Gln Asp Gln Gln Gln  Arg Leu Ile
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Ala Gly  Asp Ser Ala Pro Ser  Thr Pro Gly Ser Glu  Leu Lys Ala
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Gly Gln  Glu Leu Lys Glu Asp  Ala Arg Lys Ala Arg  Ser Gly Ser
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Pro Ser  Met Leu Ser Ala Leu  Val Pro Ser Arg Leu  Ile Arg Ala
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Val Arg  Ser Gly Ser Ile Ser  Ser Gln Thr Asn Pro  Val Pro Ser
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Ser Met  Arg Ser Ser Val Thr  Pro His Ser Pro Glu  Met Lys Gly
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Ile Ile  Gly Ser Leu Pro Ser  Pro Val Ser Ser Phe  Glu Ser Gly
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Glu Gly  Leu Leu Gln Thr Asp  Glu Glu Val Ala Gln  Ala Ser Ser
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Lys Ala  Leu Ala Leu Gln Gly  Ser Asp Thr Ala Asp  Leu Ser Arg
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Glu Ser  Ser Val Gln Ala Lys  Ser Asp Val Met Asp  Asp Leu Val
    1160                1165                1170

Ala Val  Lys Glu Glu Glu Glu  Asp Glu Thr Asp Gln  Gln Arg Leu
    1175                1180                1185

Leu Asp  Ala Ala Tyr Val Asp  Glu Tyr Val Asp Glu  Glu Asp Glu
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<212> TYPE: DNA
<213> ORGANISM: Mortierella alpina

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cgcgggtgac tctgcgccgt caacgccagg atcagagttg aaggcaggac aggagctgaa 3960

ggaggatgca aggaaggcac gatctggctc gccatcgatg ctctctgctc ttgttccatc 4020

gcggttaatc cgcgcagtga ggagtggcag catcagcagt cagaccaacc ctgtgccctc 4080

gtcgatgcgg agttcggtta caccgcattc gcccgagatg aaagggatca tcgggtcgct 4140

gccgtcacca gtgtcttcgt ttgagagcgg tgcggatgtg gtgcgtcgga tgtccattcc 4200

ctcgcctcca ccgttggagg ggctgctcca gacggatgag gaggtggctc aggcatcgag 4260

caaggcgctg gcgcttcagg gatcggacac agcagatttg agcagagaga gcagtgttca 4320

ggccaagagt gatgtgatgg acgaccttgt ggcggtcaag gaggaagagg aggacgagac 4380

cgatcagcag cggttgctgg atgcagcgta tgtggatgag tatgtggatg aggaggatga 4440

ggagggatat gatggatatg acgagcaggg tgaggatgag atggacgagg aggatgagga 4500

ggacgagtat ctggatgaga ttgaggagac tctggaggag ccgttcctgt ag 4552

<210> SEQ ID NO 11
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer MAPAH1-1-3F

<400> SEQUENCE: 11

cgccaataca ttgacgtttt cag 23

<210> SEQ ID NO 12
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer MaPAH1-1-5R

<400> SEQUENCE: 12

agttccagtc attgaactcg ggtgc 25

<210> SEQ ID NO 13
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer MaPAH1-2-3F

<400> SEQUENCE: 13

gagcccagtt gacctttgag gcattc 26

<210> SEQ ID NO 14

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<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer MaPAH1-2-5R

<400> SEQUENCE: 14

cactgagaac gagaccgtgt tggcg 25

<210> SEQ ID NO 15
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer NotI-PAH1-1-F

<400> SEQUENCE: 15

gcggccgcat gcagtccgtg ggaag 25

<210> SEQ ID NO 16
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer MaPAH1-1-10R

<400> SEQUENCE: 16

ttcttgagta gctgctgttg ttcg 24

<210> SEQ ID NO 17
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer KpnI-PAH1-F

<400> SEQUENCE: 17

ggtaccatgc agtacgtagg cagagctc 28

<210> SEQ ID NO 18
<211> LENGTH: 28
<212> TYPE: DNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer XhoI-PAH1-R

<400> SEQUENCE: 18

ctcgagttaa tcttcgaatt catcttcg 28

<210> SEQ ID NO 19
<211> LENGTH: 862
<212> TYPE: PRT
<213> ORGANISM: Saccharomyces cerevisiae

<400> SEQUENCE: 19

Met Gln Tyr Val Gly Arg Ala Leu Gly Ser Val Ser Lys Thr Trp Ser
1 5 10 15

Ser Ile Asn Pro Ala Thr Leu Ser Gly Ala Ile Asp Val Ile Val Val
20 25 30

Glu His Pro Asp Gly Arg Leu Ser Cys Ser Pro Phe His Val Arg Phe
35 40 45

Gly Lys Phe Gln Ile Leu Lys Pro Ser Gln Lys Lys Val Gln Val Phe
50 55 60

Ile Asn Glu Lys Leu Ser Asn Met Pro Met Lys Leu Ser Asp Ser Gly

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65                  70                  75                  80

Glu Ala Tyr Phe Val Phe Glu Met Gly Asp Gln Val Thr Asp Val Pro
                85                  90                  95

Asp Glu Leu Leu Val Ser Pro Val Met Ser Ala Thr Ser Ser Pro Pro
            100                 105                 110

Gln Ser Pro Glu Thr Ser Ile Leu Glu Gly Gly Thr Glu Gly Glu Gly
        115                 120                 125

Glu Gly Glu Asn Glu Asn Lys Lys Lys Glu Lys Lys Val Leu Glu Glu
    130                 135                 140

Pro Asp Phe Leu Asp Ile Asn Asp Thr Gly Asp Ser Gly Ser Lys Asn
145                 150                 155                 160

Ser Glu Thr Thr Gly Ser Leu Ser Pro Thr Glu Ser Ser Thr Thr Thr
                165                 170                 175

Pro Pro Asp Ser Val Glu Glu Arg Lys Leu Val Glu Gln Arg Thr Lys
            180                 185                 190

Asn Phe Gln Gln Lys Leu Asn Lys Lys Leu Thr Glu Ile His Ile Pro
        195                 200                 205

Ser Lys Leu Asp Asn Asn Gly Asp Leu Leu Leu Asp Thr Glu Gly Tyr
    210                 215                 220

Lys Pro Asn Lys Asn Met Met His Asp Thr Asp Ile Gln Leu Lys Gln
225                 230                 235                 240

Leu Leu Lys Asp Glu Phe Gly Asn Asp Ser Asp Ile Ser Ser Phe Ile
                245                 250                 255

Lys Glu Asp Lys Asn Gly Asn Ile Lys Ile Val Asn Pro Tyr Glu His
            260                 265                 270

Leu Thr Asp Leu Ser Pro Pro Gly Thr Pro Pro Thr Met Ala Thr Ser
        275                 280                 285

Gly Ser Val Leu Gly Leu Asp Ala Met Glu Ser Gly Ser Thr Leu Asn
    290                 295                 300

Ser Leu Ser Ser Ser Pro Ser Gly Ser Asp Thr Glu Asp Glu Thr Ser
305                 310                 315                 320

Phe Ser Lys Glu Gln Ser Ser Lys Ser Glu Lys Thr Ser Lys Lys Gly
                325                 330                 335

Thr Ala Gly Ser Gly Glu Thr Glu Lys Arg Tyr Ile Arg Thr Ile Arg
            340                 345                 350

Leu Thr Asn Asp Gln Leu Lys Cys Leu Asn Leu Thr Tyr Gly Glu Asn
        355                 360                 365

Asp Leu Lys Phe Ser Val Asp His Gly Lys Ala Ile Val Thr Ser Lys
    370                 375                 380

Leu Phe Val Trp Arg Trp Asp Val Pro Ile Val Ile Ser Asp Ile Asp
385                 390                 395                 400

Gly Thr Ile Thr Lys Ser Asp Ala Leu Gly His Val Leu Ala Met Ile
                405                 410                 415

Gly Lys Asp Trp Thr His Leu Gly Val Ala Lys Leu Phe Ser Glu Ile
            420                 425                 430

Ser Arg Asn Gly Tyr Asn Ile Leu Tyr Leu Thr Ala Arg Ser Ala Gly
        435                 440                 445

Gln Ala Asp Ser Thr Arg Ser Tyr Leu Arg Ser Ile Glu Gln Asn Gly
    450                 455                 460

Ser Lys Leu Pro Asn Gly Pro Val Ile Leu Ser Pro Asp Arg Thr Met
465                 470                 475                 480

Ala Ala Leu Arg Arg Glu Val Ile Leu Lys Lys Pro Glu Val Phe Lys
                485                 490                 495
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Ile Ala Cys Leu Asn Asp Ile Arg Ser Leu Tyr Phe Glu Asp Ser Asp
            500                 505                 510

Asn Glu Val Asp Thr Glu Glu Lys Ser Thr Pro Phe Phe Ala Gly Phe
        515                 520                 525

Gly Asn Arg Ile Thr Asp Ala Leu Ser Tyr Arg Thr Val Gly Ile Pro
    530                 535                 540

Ser Ser Arg Ile Phe Thr Ile Asn Thr Glu Gly Glu Val His Met Glu
545                 550                 555                 560

Leu Leu Glu Leu Ala Gly Tyr Arg Ser Ser Tyr Ile His Ile Asn Glu
                565                 570                 575

Leu Val Asp His Phe Phe Pro Pro Val Ser Leu Asp Ser Val Asp Leu
            580                 585                 590

Arg Thr Asn Thr Ser Met Val Pro Gly Ser Pro Pro Asn Arg Thr Leu
        595                 600                 605

Asp Asn Phe Asp Ser Glu Ile Thr Ser Gly Arg Lys Thr Leu Phe Arg
    610                 615                 620

Gly Asn Gln Glu Glu Lys Phe Thr Asp Val Asn Phe Trp Arg Asp Pro
625                 630                 635                 640

Leu Val Asp Ile Asp Asn Leu Ser Asp Ile Ser Asn Asp Asp Ser Asp
                645                 650                 655

Asn Ile Asp Glu Asp Thr Asp Val Ser Gln Gln Ser Asn Ile Ser Arg
            660                 665                 670

Asn Arg Ala Asn Ser Val Lys Thr Ala Lys Val Thr Lys Ala Pro Gln
        675                 680                 685

Arg Asn Val Ser Gly Ser Thr Asn Asn Asn Glu Val Leu Ala Ala Ser
    690                 695                 700

Ser Asp Val Glu Asn Ala Ser Asp Leu Val Ser Ser His Ser Ser Ser
705                 710                 715                 720

Gly Ser Thr Pro Asn Lys Ser Thr Met Ser Lys Gly Asp Ile Gly Lys
                725                 730                 735

Gln Ile Tyr Leu Glu Leu Gly Ser Pro Leu Ala Ser Pro Lys Leu Arg
            740                 745                 750

Tyr Leu Asp Asp Met Asp Asp Glu Asp Ser Asn Tyr Asn Arg Thr Lys
        755                 760                 765

Ser Arg Arg Ala Ser Ser Ala Ala Ala Thr Ser Ile Asp Lys Glu Phe
    770                 775                 780

Lys Lys Leu Ser Val Ser Lys Ala Gly Ala Pro Thr Arg Ile Val Ser
785                 790                 795                 800

Lys Ile Asn Val Ser Asn Asp Val His Ser Leu Gly Asn Ser Asp Thr
                805                 810                 815

Glu Ser Arg Arg Glu Gln Ser Val Asn Glu Thr Gly Arg Asn Gln Leu
            820                 825                 830

Pro His Asn Ser Met Asp Asp Lys Asp Leu Asp Ser Arg Val Ser Asp
        835                 840                 845

Glu Phe Asp Asp Asp Glu Phe Asp Glu Asp Glu Phe Glu Asp
    850                 855                 860


<210> SEQ ID NO 20
<211> LENGTH: 891
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 20

Met Asn Tyr Val Gly Gln Leu Ala Gly Gln Val Phe Val Thr Val Lys
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1               5                   10                  15

Glu Leu Tyr Lys Gly Leu Asn Pro Ala Thr Leu Ser Gly Cys Ile Asp
            20                  25                  30

Ile Ile Val Ile Arg Gln Pro Asn Gly Ser Leu Gln Cys Ser Pro Phe
        35                  40                  45

His Val Arg Phe Gly Lys Met Gly Val Leu Arg Ser Arg Glu Lys Val
    50                  55                  60

Val Asp Ile Glu Ile Asn Gly Glu Ser Val Asp Leu His Met Lys Leu
65                  70                  75                  80

Gly Asp Asn Gly Glu Ala Phe Phe Val Gln Glu Thr Asp Asn Asp Gln
                85                  90                  95

Glu Ile Ile Pro Met Tyr Leu Ala Thr Ser Pro Ile Leu Ser Glu Gly
            100                 105                 110

Ala Ala Arg Met Glu Ser Gln Leu Lys Arg Asn Ser Val Asp Arg Ile
        115                 120                 125

Arg Cys Leu Asp Pro Thr Thr Ala Ala Gln Gly Leu Pro Pro Ser Asp
    130                 135                 140

Thr Pro Ser Thr Gly Ser Leu Gly Lys Lys Arg Arg Lys Arg Arg Arg
145                 150                 155                 160

Lys Ala Gln Leu Asp Asn Leu Lys Arg Asp Asp Asn Val Asn Ser Ser
                165                 170                 175

Glu Asp Glu Asp Met Phe Pro Ile Glu Met Ser Ser Asp Glu Asp Thr
            180                 185                 190

Ala Pro Met Asp Gly Ser Arg Thr Leu Pro Asn Asp Val Pro Pro Phe
        195                 200                 205

Gln Asp Asp Ile Pro Lys Glu Asn Phe Pro Ser Ile Ser Thr His Pro
    210                 215                 220

Gln Ser Ala Ser Tyr Pro Ser Ser Asp Arg Glu Trp Ser Pro Ser Pro
225                 230                 235                 240

Ser Pro Ser Gly Ser Arg Pro Ser Thr Pro Lys Ser Asp Ser Glu Leu
                245                 250                 255

Val Ser Lys Ser Ala Asp Arg Leu Thr Pro Lys Asn Asn Leu Glu Met
            260                 265                 270

Leu Trp Leu Trp Gly Glu Leu Pro Gln Ala Ala Lys Ser Ser Ser Pro
        275                 280                 285

His Lys Met Lys Glu Ser Ser Pro Leu Gly Ser Arg Lys Thr Pro Asp
    290                 295                 300

Lys Met Asn Phe Gln Ala Ile His Ser Glu Ser Ser Asp Thr Phe Ser
305                 310                 315                 320

Asp Gln Ser Pro Thr Met Ala Arg Gly Leu Leu Ile His Gln Ser Lys
                325                 330                 335

Ala Gln Thr Glu Met Gln Phe Val Asn Glu Glu Asp Leu Glu Ser Leu
            340                 345                 350

Gly Ala Ala Ala Pro Pro Ser Pro Val Ala Glu Glu Leu Lys Ala Pro
        355                 360                 365

Tyr Pro Asn Thr Ala Gln Ser Ser Ser Lys Thr Asp Ser Pro Ser Arg
    370                 375                 380

Lys Lys Asp Lys Arg Ser Arg His Leu Gly Ala Asp Gly Val Tyr Leu
385                 390                 395                 400

Asp Asp Leu Thr Asp Met Asp Pro Glu Val Ala Ala Leu Tyr Phe Pro
                405                 410                 415

Lys Asn Gly Asp Pro Gly Gly Leu Pro Lys Gln Ala Ser Asp Asn Val
            420                 425                 430
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```
Ala Arg Ser Ala Asn Gln Ser Pro Gln Ser Val Gly Gly Ser Gly Ile
        435                 440                 445

Asp Ser Gly Val Glu Ser Thr Ser Asp Ser Leu Arg Asp Leu Pro Ser
    450                 455                 460

Ile Ala Ile Ser Leu Cys Gly Gly Leu Ser Asp His Arg Glu Ile Thr
465                 470                 475                 480

Lys Asp Ala Phe Leu Glu Gln Ala Val Ser Tyr Gln Gln Phe Ala Asp
                485                 490                 495

Asn Pro Ala Ile Ile Asp Asp Pro Asn Leu Val Val Lys Val Gly Asn
            500                 505                 510

Lys Tyr Tyr Asn Trp Thr Thr Ala Ala Pro Leu Leu Leu Ala Met Gln
        515                 520                 525

Ala Phe Gln Lys Pro Leu Pro Lys Ala Thr Val Glu Ser Ile Met Arg
    530                 535                 540

Asp Lys Met Pro Lys Lys Gly Gly Arg Trp Trp Phe Ser Trp Arg Gly
545                 550                 555                 560

Arg Asn Ala Thr Ile Lys Glu Glu Ser Lys Pro Glu Gln Cys Leu Thr
                565                 570                 575

Gly Lys Gly His Asn Thr Gly Glu Gln Pro Ala Gln Leu Gly Leu Ala
            580                 585                 590

Thr Arg Ile Lys His Glu Ser Ser Ser Ser Asp Glu Glu His Ala Ala
        595                 600                 605

Ala Lys Pro Ser Gly Ser Ser His Leu Ser Leu Leu Ser Asn Val Ser
    610                 615                 620

Tyr Lys Lys Thr Leu Arg Leu Thr Ser Glu Gln Leu Lys Ser Leu Lys
625                 630                 635                 640

Leu Lys Asn Gly Pro Asn Asp Val Val Phe Ser Val Thr Thr Gln Tyr
                645                 650                 655

Gln Gly Thr Cys Arg Cys Glu Gly Thr Ile Tyr Leu Trp Asn Trp Asp
            660                 665                 670

Asp Lys Val Ile Ile Ser Asp Ile Asp Gly Thr Ile Thr Arg Ser Asp
        675                 680                 685

Thr Leu Gly His Ile Leu Pro Thr Leu Gly Lys Asp Trp Thr His Gln
    690                 695                 700

Gly Ile Ala Lys Leu Tyr His Lys Val Ser Gln Asn Gly Tyr Lys Phe
705                 710                 715                 720

Leu Tyr Cys Ser Ala Arg Ala Ile Gly Met Ala Asp Met Thr Arg Gly
                725                 730                 735

Tyr Leu His Trp Val Asn Glu Arg Gly Thr Val Leu Pro Gln Gly Pro
            740                 745                 750

Leu Leu Leu Ser Pro Ser Ser Leu Phe Ser Ala Leu His Arg Glu Val
        755                 760                 765

Ile Glu Lys Lys Pro Glu Lys Phe Lys Val Gln Cys Leu Thr Asp Ile
    770                 775                 780

Lys Asn Leu Phe Phe Pro Asn Thr Glu Pro Phe Tyr Ala Ala Phe Gly
785                 790                 795                 800

Asn Arg Pro Ala Asp Val Tyr Ser Tyr Lys Gln Val Gly Val Ser Leu
                805                 810                 815

Asn Arg Ile Phe Thr Val Asn Pro Lys Gly Glu Leu Val Gln Glu His
            820                 825                 830

Ala Lys Thr Asn Ile Ser Ser Tyr Val Arg Leu Cys Glu Val Val Asp
        835                 840                 845
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His Val Phe Pro Leu Leu Lys Arg Ser His Ser Cys Asp Phe Pro Cys
    850                 855                 860

Ser Asp Thr Phe Ser Asn Phe Thr Phe Trp Arg Glu Pro Leu Pro Pro
865                 870                 875                 880

Phe Glu Asn Gln Asp Met His Ser Ala Ser Ala
                885                 890
```

The invention claimed is:

1. A cDNA or recombinant vector comprising:

(a) a nucleotide sequence encoding a protein that consists of an amino acid sequence having deletion, substitution, or addition of 1-50 amino acids in the amino acid sequence set forth in SEQ ID NO: 2 and has a phosphatidic acid phosphatase activity; or

(b) a nucleotide sequence encoding a protein that consists of an amino acid sequence having an identity of 95% or more with the amino acid sequence set forth in SEQ ID NO: 2 and has a phosphatidic acid phosphatase activity,

wherein 100 amino acids at the N-terminus and the DXDX(T/V) catalytic site motif in the protein are identical to SEQ ID NO: 2.

2. A cDNA or recombinant vector comprising:

(a) a nucleotide sequence encoding a protein that consists of an amino acid sequence having deletion, substitution, or addition of 1-50 amino acids in the amino acid sequence set forth in SEQ ID NO: 2 and has an activity that enhances generation of diacylglycerol (DG) and/or triglyceride (TG) from phosphatidic acid (PA) in a PAH1-deficient yeast strain; or

(b) a nucleotide sequence encoding a protein that consists of an amino acid sequence having an identity of 95% or more with the amino acid sequence set forth in SEQ ID NO: 2 and has an activity that enhances generation of DG and/or TG from PA in a PAH1-deficient yeast strain,

wherein 100 amino acids at the N-terminus and the DXDX(T/V) catalytic site motif in the protein are identical to SEQ ID NO: 2.

3. A cDNA or recombinant vector comprising a nucleic acid sequence according to any one of (a) to (d) below:

(a) the nucleotide sequence set forth in SEQ ID NO: 1;

(b) a nucleotide sequence encoding a protein consisting of the amino acid sequence set forth in SEQ ID NO: 2;

(c) the nucleotide sequence set forth in SEQ ID NO: 4; and

(d) the nucleotide sequence set forth in SEQ ID NO: 5.

4. An isolated transformant transformed with the recombinant vector according to claim **1**.

5. A method for producing a lipid composition, comprising:

culturing the transformant according to claim **4**; and

collecting a lipid from the culture,

wherein the lipid comprises diacylglycerol (DG) and/or triglyceride (TG).

* * * * *